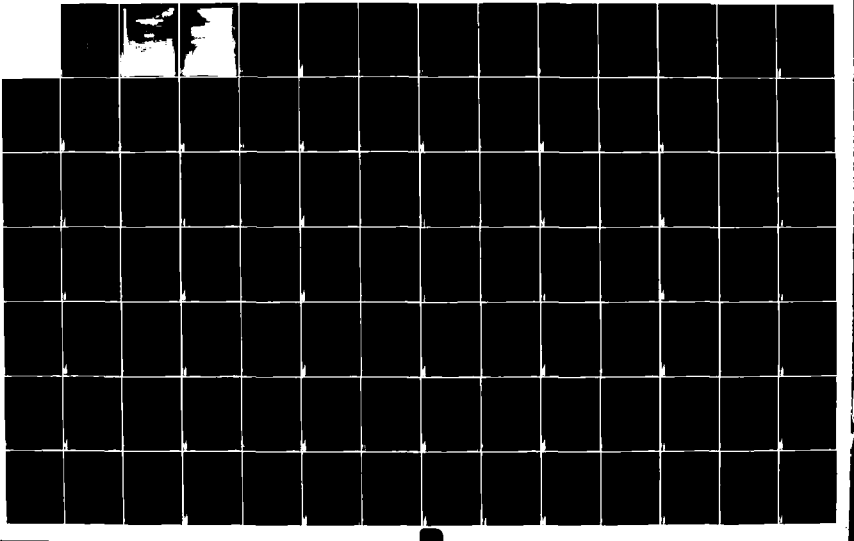
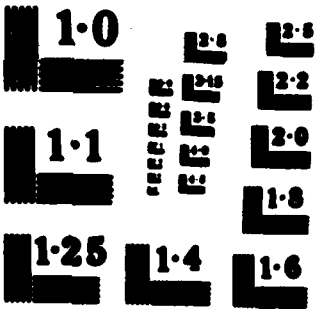
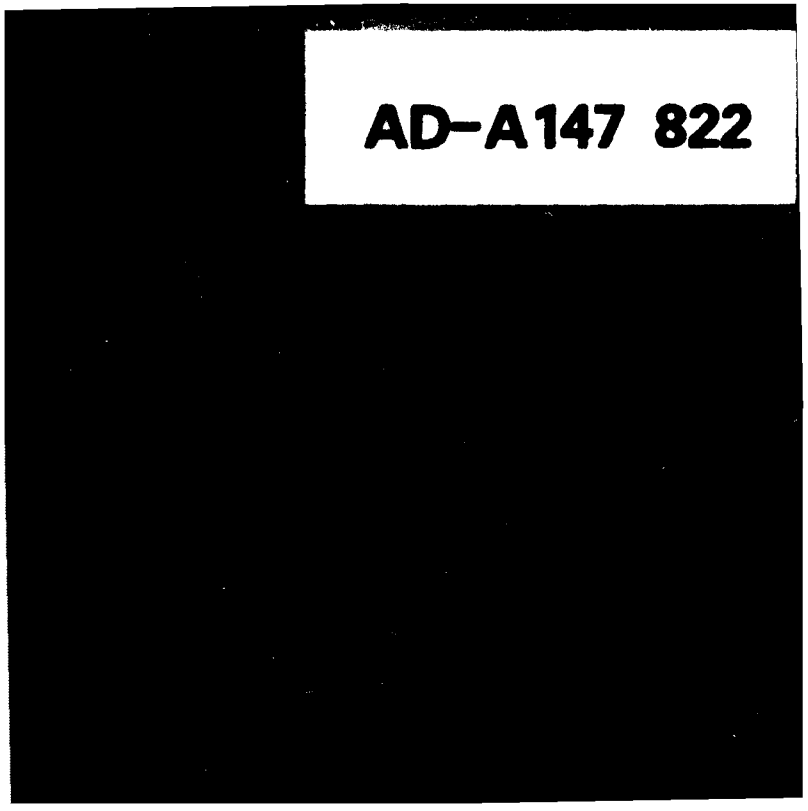
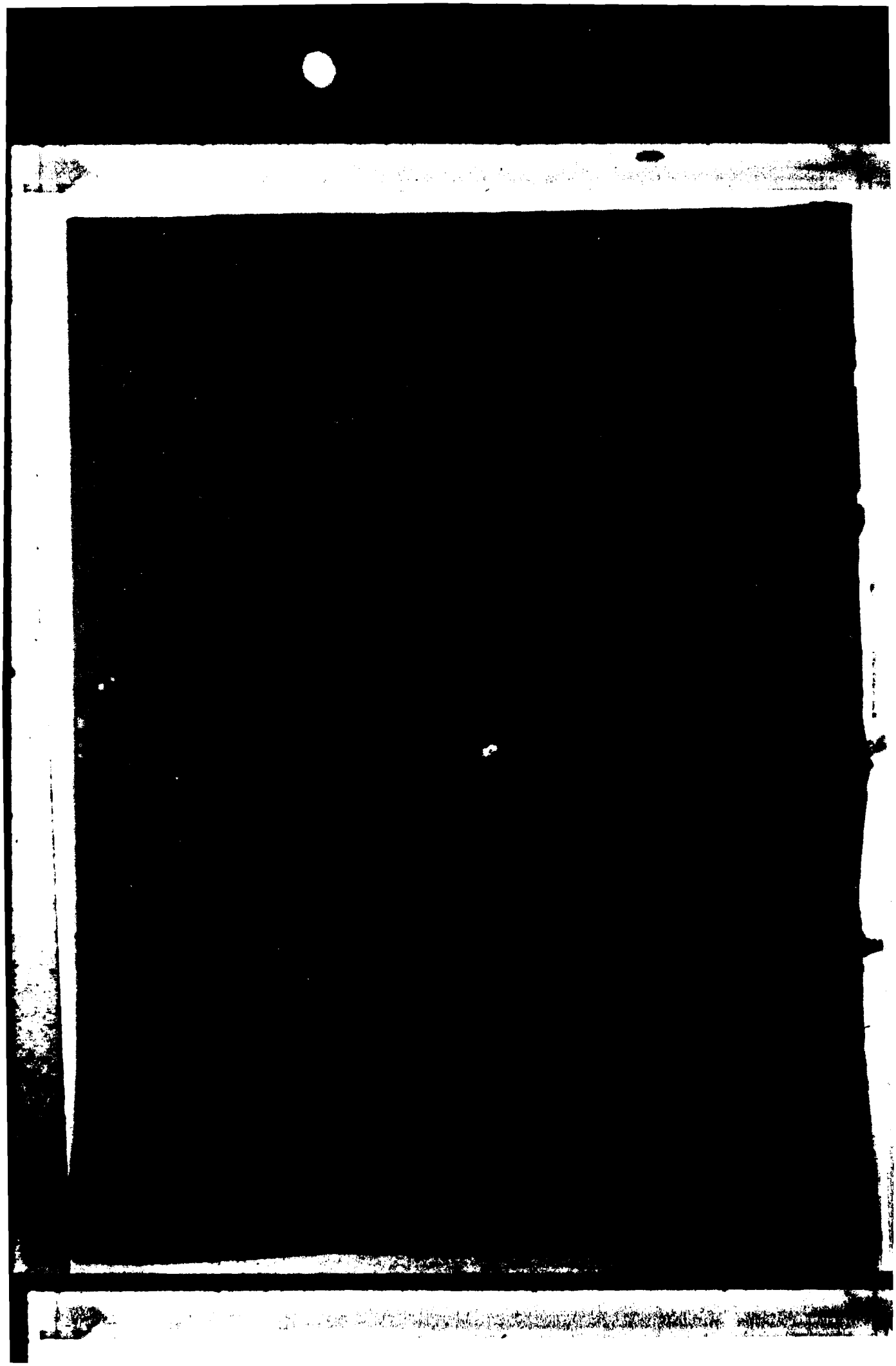
AD A147 822 CHEMICAL PROTECTION AGAINST IONIZING RADIATION(U) 1/2
OREGON STATE UNIV CORVALLIS J C LIVESEY ET AL. AUG 84
UCRL-15644 EMW-E-0883

UNCLASSIFIED F/G 6/18 NL




1·0
1·1
1·25
1·4
1·6
1·8
2·0
2·2
2·5


AD-A147 822



Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A147822	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Chemical Protection Against Ionizing Radiation	5. TYPE OF REPORT & PERIOD COVERED Final Report
	6. PERFORMING ORG. REPORT NUMBER UCRL-15844
7. AUTHOR(s) J. C. Livesey, D. J. Reed, L. F. Adamson	8. CONTRACT OR GRANT NUMBER(s) P.O. 1369905
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Health Sciences Center Oregon State University Corvallis, OR 97331	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Work Unit 2432D
11. CONTROLLING OFFICE NAME AND ADDRESS Federal Emergency Management Agency Washington, D.C. 20472	12. REPORT DATE August 1984
	13. NUMBER OF PAGES 151
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Lawrence Livermore National Laboratory P. O. Box 808 Livermore, CA 94550	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release; Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Chemical Radiation Protection
Radioprotectors
Literature Review
Pulse Radiolysis
Radiation Chemistry
Radiation Biology
Therapy of Radiation Injury

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The scientific literature on radiation-protective drugs is reviewed. Emphasis is placed on the mechanisms involved in determining the sensitivity of biological material to ionizing radiation and mechanisms of chemical radioprotection. In Section I, the types of radiation are described and the effects of ionizing radiation on biological systems are reviewed. The effects of ionizing radiation are briefly contrasted with the effects of non-ionizing radiation.

continued

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. Abstract (continued)

Section II reviews the contributions of various natural factors which influence the inherent radiosensitivity of biological systems. Included in the list of these factors are water, oxygen, thiols, vitamins and antioxidants. Brief attention is given to the model describing competition between oxygen and natural radioprotective substances (principally, thiols) in determining the net cellular radiosensitivity.

Several theories of the mechanism(s) of action of radioprotective drugs are described in Section III. These mechanisms include the production of hypoxia, detoxication of radiochemical reactive species, stabilization of the radiobiological target and the enhancement of damage repair processes. Section IV describes the current strategies for the treatment of radiation injury. Likely areas in which fruitful research might be performed are described in Section V.

Appendices are devoted to lists of currently-funded research projects involving chemical radiation protection and a brief compendium of compounds which have been tested for radioprotective activity.

DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS
and
ENVIRONMENTAL HEALTH SCIENCES CENTER
OREGON STATE UNIVERSITY
CORVALLIS, OREGON 97331

CHEMICAL PROTECTION AGAINST IONIZING RADIATION

by

John C. Livesey
Donald J. Reed
Lucile F. Adamson

FINAL REPORT
AUGUST 1984

Prepared for

FEDERAL EMERGENCY MANAGEMENT AGENCY
Washington D.C.
20472

Acknowledgements

We would like to acknowledge the assistance of several individuals whose efforts contributed to the completion of this report. A number of researchers in the fields of radiation chemistry, biology and oncology shared published and unpublished information which contributed to this report. Included in this generous group are: G. E. Adams, K.-D. Asmus, B. H. J. Bielski, C. Borek, J. Denekamp, E. Fickweiler, C. Koch, L. Milas, M. Paterson, J. A. Raleigh, L. Revesz, E. Riklis, C. A. Waldren, and H. R. Withers.

A large amount of credit goes to Nanette Cardon for her indefatigable efforts in maintaining the organization, filing and computer searching of the many bytes of information on radiation protection which we have collected during the course of this project. Her patient, diligent and efficient typing and printing of this manuscript is also gratefully appreciated.

Preface

The field of radiation protection carries a certain amount of stigma from the public about the sources of radiation, its effects, and uses. Certainly, few would fail to acknowledge the beneficial effects of diagnostic radiology and radiation therapy. However, the public concern over low levels of radiation, coupled with concern over nuclear issues in general, is pressing and likely to continue. In this atmosphere of concern, the topic of chemical protection against ionizing radiation is viewed by many people as a means of skirting or minimizing the hazards of exposure to ionizing radiation, or as a way to make increased exposure more acceptable. However, radiation exposure is with us as a fact of life, and accidents with ionizing radiation sources do occur, as recently happened in Juarez, Mexico (Marshall, E., Science 1984 223, 1152-1154). The presence of nuclear power plants, and their finite lifetime, will result in occupational exposure to radiation as they are decommissioned early in the next century. As one looks at the types of radioprotectors and their effectiveness, the notion that radiation exposure will increase because of reliance on chemical means of preventing the damage of ionizing radiation becomes quite implausible. Certainly, no chemical means can prevent or even significantly reduce the horrors of strategic use of nuclear weaponry or the large scale accidential emission of radiation from power sources. However, benefits from effective radioprotectors can be realized. Some of the hazards of modest accidential exposure to radioactive materials (such as the recent Juarez incident) may be lessened by the application of radioprotectors or effective treatments for "radiation sickness." Individuals who are genetically highly susceptible to natural radiation sources may derive benefit from research on chemical radioprotection. The clinical use of radioprotective agents as prophylactic adjunct therapy in radiation oncology has been a continuing goal of radiation oncologists for many years. It is for these purposes that we have reviewed the literature on chemical protection against ionizing radiation and submit this report on our efforts.

Summary

The scientific literature on radiation-protective drugs is reviewed. Emphasis is placed on the mechanisms involved in determining the sensitivity of biological material to ionizing radiation and mechanisms of chemical radioprotection. In Section I, the types of radiation are described and the effects of ionizing radiation on biological systems are reviewed. The effects of ionizing radiation are briefly contrasted with the effects of non-ionizing radiation.

Section II reviews the contributions of various natural factors which influence the inherent radiosensitivity of biological systems. Included in the list of these factors are water, oxygen, thiols, vitamins and antioxidants. Brief attention is given to the model describing competition between oxygen and natural radioprotective substances (principally, thiols) in determining the net cellular radiosensitivity.

Several theories of the mechanism(s) of action of radioprotective drugs are described in Section III. These mechanisms include the production of hypoxia, detoxication of radiochemical reactive species, stabilization of the radiobiological target and the enhancement of damage repair processes. Section IV describes the current strategies for the treatment of radiation injury. Likely areas in which fruitful research might be performed are described in Section V.

Appendices are devoted to lists of currently-funded research projects involving chemical radiation protection and a brief compendium of compounds which have been tested for radioprotective activity.

TABLE OF CONTENTS

I. Effects of Radiation on Biological Systems.

A. Introduction.

In this section, some of the general terms and concepts dealing with radiation and with radiation injury to biological systems are introduced. In Section II, radioprotection and radioprotective agents are discussed in more detail. The related topics of radiosensitization and of non-ionizing radiation effects are considered only to the extent that such consideration is relevant to chemical protection against ionizing radiation. Among the reviews which have been published on the general subject of radiation biology are those of Dertinger and Jung [120] and Pizzarello and Witcofski [352], which were used as sources of much of the introductory material in this section.

B. Types of Radiation.

Table 1 lists forms of radiation that are pertinent to this review. Those forms which consist of energetic particles and certain of the electromagnetic spectrum rays (cosmic, gamma, and X-rays) are commonly designated as ionizing radiation. When these types of radiation penetrate biological tissue (or other forms of matter), some of the neutral atoms along the paths of penetration are converted to ions. At the other end of the electromagnetic spectrum are microwaves and radiowaves which are characterized as non-ionizing radiation. Ultraviolet wavelengths are intermediate, in both frequency and biological effects, between ionizing and non-ionizing radiation.

C. Effects of Ionizing Radiation at the Atomic and Molecular Levels.

The physical and chemical lesions produced when ionizing radiation penetrates biological tissue result from the transfer of radiation energy to the tissue. The first tissue/radiation interaction is at the physical level (energy transfer to some tissue component) with secondary and higher order effects at the physico-chemical, chemical, and biological levels. As approximate orders of magnitude the relative time required for these stages is: physical = 10^{-13} sec., physico-chemical = 10^{-10} sec., chemical = 10^{-6} sec., and biological = seconds to years.

Whether one considers direct effects (terminal absorption of the energy of an incoming particle or photon by DNA or another functional biomolecule, with resultant chemical change of that molecule) or indirect effects (damaging energy transfer to the biomolecule only after intermediate absorption

TABLE 1: SOME TYPES OF RADIATION

TYPE OF RADIATION		SOURCES	I/NI*
Particulate:			
Electrons (β^- particles)		Radioactive decay	I
Positrons (β^+ particles)		Radioactive decay	I
Protons		Cyclotron; Van de Graff generator	I
Neutrons		Nuclear fission, Cyclotron	I
Helium nuclei	(α particles)	Radioactive decay	I
Nuclei of heavier elements		Particle Accelerators	I
Electromagnetic	(wavelength)		
Cosmic rays	(5×10^{-5} nm)	Stars	I
Gamma rays	(5×10^{-4} - 1.4×10^{-1} nm)	Radioactive decay	I
X-rays	(1×10^{-2} - 10 nm)	X-ray machine	I
Ultraviolet	(≈ 280-400 nm)	Sunlight, artificial sources	Int
Microwaves	(≈ 0.3-300 cm)	Radio, television, other transmitters, other artificial EM fields	NI
Radiowaves	(> 0.3 cm)	Communications; cosmic sources	NI

* I, Ionizing; NI, Nonionizing; Int, Intermediate.

and transfers by diffusible ions and radicals), the basic cause of radiation damage is the same: disruptive absorption of the energy of photons and/or energized subatomic particles.

1. Modes of Energy Transfer.

For the most part, primary energy transfer occurs through ejection or excitation of orbital electrons in atoms along the radiation path, although some transfer occurs through direct collision with the nuclei of those atoms. The paths of low-mass particles, such as electrons, will be irregular since these particles are subject to scattering and deflection by collisions with electrons of the medium.

Ionization: In ionizing events, the passage of an energetic particle (or a photon) causes ejection of an electron from an atom with the resultant creation of a positive and negative ion pair. This is the principal means by which the energy of ionizing radiation (whether particulate or electromagnetic) is transferred to biological tissues. Water is most frequently the molecule from which the electron is expelled because of its preponderance in most biological tissues. The "water radicals" that result are the principal

agents which re-transfer the absorbed radiation energy to "target" biomolecules.

Excitation: In addition to ionization, a significant fraction of the energy of energetic particles or of electromagnetic radiation is dissipated by electron excitation. In this process, an outer electron of a target atom absorbs enough energy to move to a higher energy state but remains associated with the atom.

Collision: Whereas X-rays, γ-rays, ultraviolet radiation, and charged particles interact chiefly with intercepted atoms to eject or excite orbital electrons from these atoms, the energy of neutron radiation is absorbed chiefly by direct collision with nuclei, causing the ejection of disintegration fragments (such as neutrons, protons, or alpha particles) bearing the transferred kinetic energy of the colliding neutrons. In biological tissue with its preponderance of hydrogen atoms, the production of high-speed protons (hydrogen nuclei) is the most frequent consequence of these collisions. Nuclear disintegration can also be produced by high energy photons. Photons or electrons can interact with target nuclei to produce secondary X-ray emissions.

Indirect Energy Transfer: Whether the radiation energy is initially absorbed by ionization, excitation, or collision, a secondary production of free protons, electrons, ions, free radicals, or photons can occur as the energy is re-transferred to other atoms and molecules. The disintegration of target nuclei which have become unstable due to ejection or absorption of particles also contributes to the secondary production of ions by releasing a shower of energetic particles.

Among the products of the energy-absorption process, "free radicals" are of particular significance. A free radical is an atom with a single unpaired orbital electron, atomic hydrogen being the simplest example. A free radical is more long-lived than an ion pair and has a high probability of reacting with another atom either by pairing of its unpaired electron with an electron of that other atom, by releasing its unpaired electron to the other atom, or by capturing an electron from the other atom. Each of these interactions can in turn create additional ions or free radicals. Most of the radiation damage to organic molecules is associated with such chains of secondary free-radical interactions.

2. Significant Variables.

The nature of the effects which will occur when ionizing radiation penetrates tissue depends upon a multiplicity of variables. Some of the more significant ones are listed here.

Type of radiation: The results of the interaction between radiation and tissue will be influenced by whether the radiation is particulate or non-particulate and, if the former, by the size and charge of the energetic particle. For example, because neutrons are not slowed or deflected by the negative charge or the much smaller mass of encountered electrons, their energy is more likely to be absorbed by direct nuclear collisions. The energy of the smaller charged particles of β-rays, on the other hand, will be largely dissipated in ionization or excitation interactions with orbital electrons. Interactions of heavy ions from particle accelerators are influenced by both their charge and their mass. The more highly charged a particle is, the more frequent will be its interactions and the greater will be the density of ions produced along its track. The more massive it is, the slower it will be (at a given energy level) and the greater will be the density of ionization events. The energy of massless ultraviolet, X- and γ-rays is transferred in photon/electron collisions and biological damage produced by this radiation is due almost entirely to the action of the free radicals and ions produced.

Particle velocity: The density of ions produced along the track of an ionized particle is influenced by its velocity. The slower it is, the greater will be the number of ions produced per millimeter of track. For this reason, ionization density increases continually along a particle track as the particle is gradually slowed by incremental energy transfers. The most abundant ion production occurs just before the particle comes to rest.

Wavelength: For non-particulate radiation, the greater its energy (i.e., the shorter its wavelength), the greater is its potential to support damaging energy transfer events in tissue.

LET/RBE: Linear Energy Transfer, or LET, is a measure of the amount of energy released (by any mechanism) per micron of track of any ionizing radiation and is an indicator of the level of biological disruption to be expected. Biological damage is greater and postirradiation recovery is slower [324] for high-LET than for low-LET radiation. LET is determined in part by the variables already mentioned: it varies with the kinetic energy level

(velocity and size) and charge of radiation particles and with the wavelength (energy) of electromagnetic radiation. Since particle velocity decreases continuously along the particle path, LET also changes continuously, increasing as the particle slows until it falls abruptly to zero when the particle comes to rest. Because LET varies for different types of radiation, biological effect is sometimes expressed in terms of relative biological effectiveness (RBE), which is a ratio of the biological disruption from the radiation in question to that from some reference radiation (usually γ-rays).

Dose: Biological damage from ionizing radiation increases with the dose (i.e. the amount of radiation energy absorbed). Standard units of absorbed dose include:

the rad (R) (deposition of 100 ergs per gram of tissue),
the Gray (Gy) (100 rads, or 1 joule per kilogram of medium),
the rem (the rad corrected for the radiation quality under consideration), and
the Sievert (100 rem).

Dose rate: The extent of biological damage can also vary with the rate at which a given dose is administered (absorbed), especially for low-LET radiations. As will be discussed below, biological tissues possess some capability of repairing sub-lethal damage from ionizing radiation. For low-LET radiation (that tends to produce sub-lethal damage to cells) and low dose rates or divided doses, repair mechanisms may to some extent counteract radiation damage as it occurs whereas at low-LET/high dose-rates, the repair capacity can be overwhelmed. With high-LET radiation, where damage is more likely to be non-repairable, dose rate is less influential.

Tissue and field: Some tissues are more radiosensitive than others. Proliferating cells (e.g. hemopoietic stem cells) are more sensitive than non-proliferating cells, with the period of chromosome formation being most sensitive and the period of onset of DNA synthesis being relatively resistant. Some types of damage are produced at lower doses than are others, and the dose required for a given effect is influenced by the proportion of the tissue or organism (the "field") that is irradiated. There is also a genetic component to "radioresistance". For instance, some types of microorganisms show much greater radioresistance than others. Repair capability is one of many factors that contribute to variations in sensitivity among tissues or organisms.

Modifiers: Modifiers of the radiobiological response to ionizing radiation must be included in the list of variables governing the nature of radiation injury. Both sensitizers (e.g., oxygen) and protectors (e.g., glutathione, tocopherol) may be present and the net effect of the presence of modifiers will be determined by the type of modifier present, their concentration and relative potency. These topics are discussed more fully in §II.

3. Critical Radiation Targets.

Much effort in the field of radiobiology has been devoted to the identification of the critical radiation target whose inactivation leads to cell death. Whereas recent observations on this problem suggest that the critical factor may be the interrelationships between several targets [108], some evidence is presented below for the idea that individual targets can be identified as being of overriding importance for cell inactivation.

Nucleic Acids: A number of lines of evidence show that DNA is a critical target for radiation damage. Whereas other molecules and structures, such as enzymes and membranes, can be damaged by radiation, effects on DNA are more likely to have negative consequences for the organism. A radiation-induced defect in only one molecule of DNA can theoretically bring about the death of the cell, the death of the entire organism (if the defect is a carcinogenic one) or a severe abnormality in the progeny of the damaged cell or organism (if the defect is a mutagenic one). One would not expect the effects of a single lesion in a non-nucleic acid molecule or structure to have such significant effects.

Reasons for the identification of DNA as the critical target molecule for ionizing radiation are briefly summarized in Chapter 14 of the monograph by Dertinger and Jung [120]. Perhaps the most persuasive evidence is the correlation of radiation sensitivity (in viruses, bacteria, yeast, cultured cells, and plants) with DNA content, rather than with the size of the cell or some other variable.

Ionizing radiation brings about changes in both the physical/chemical and functional characteristics of DNA [216]. Radiation-induced free radicals and ions chemically disrupt the DNA molecule in a number of ways. Atomic hydrogen produced by water radiolysis attacks the double bonds of the purine and pyrimidine bases, and can thus produce altered DNA structures. Under aerobic conditions, the hydroxyl radical attacks the 5-6 purine double bonds

to produce unstable hydroxy peroxides. Radicals produced during water radiolysis (hydrogen and hydroxyl free radicals and the aqueous electron) also attack the deoxyribosyl component of DNA, producing chain breaks. Other chemical effects include breaking of hydrogen bonds which produce conformational changes, crosslinking of DNA with DNA or protein, and the removal of the bases.

Functional evidence of radiation-induced degradation of DNA is also seen, not only in the DNA-mediated production of mutations, birth defects, arrested cell division, and cancer (see below) but also with the loss of a number of other basic DNA functions as summarized by Dertinger and Jung [120]. These effects include radiation-induced decrease or cessation of DNA/RNA synthesis, loss of infectivity of irradiated bacteriophage, loss of bacterial transformation ability, dose dependent failure of irradiated DNA in its action as a primer for nucleic acid synthesis when incubated with DNA or RNA polymerase, diminished in-vitro hybridization between irradiated DNA and messenger RNA, loss of enzyme induction capacity in bacteria, and inactivation of viruses.

Ionizing radiation has also been shown to disrupt RNA functions in whole cells and cell-free preparations. The functions of messenger RNA, transfer RNA, and ribosomal RNA are inhibited, as was reviewed briefly by Dertinger and Jung [120]. However, RNA is more radioresistant than DNA, and transfer RNA is more resistant than messenger RNA, when measured by functional parameters. These sensitivity relationships may be explained by the relative sizes (target sizes) of the nucleic acid molecules. It is partly on the basis of these relative sensitivities that the critical event in radiation-induced cell death is considered by most workers to be interference with the function of DNA.

Membranes: A number of lines of evidence indicate that radiation also damages membranes and membrane function. Peroxidation of membrane lipids and lipids of model membranes has been reported by various groups [252,251,186, 371,369,370,491]. Irradiated yeast cells show increased amino acid influx and loss of membrane sulfhydryl groups without survival being affected [237]. The increased radiosensitivity of embryonic cells during the period of gastrulation is suggested by Dertinger and Jung [120] to be due to an effect on cell membranes. Jozwiak [229] reported increased osmotic fragility

of irradiated porcine erythrocytes. Schuurhuis [403] showed that X-irradiation of human erythrocytes or ghosts produced some cross-linking of membrane proteins and alteration of the cell shape.

The relationship between lipid peroxidation of model or cellular membranes and radiosensitivity of organisms is not well established. Attempts to define this relationship by altering the composition of membrane lipids have produced somewhat equivocal results [138,485]. Incorporation of polyunsaturated fatty acids (PUFA) into the membranes of mouse fibroblasts did not alter the radiosensitivity of these cells [485]; although lipid peroxidation was not measured in this study, a significant decrease in the membrane fluidity was observed when cells were supplemented with arachadonic acid. Similarly, cells of the prokaryote Acholeplasma laidlawii exhibit similar radiation survival characteristics when grown in the presence or absence of high concentrations of dilineoylphosphatidylcholine and irradiated in air at 5 Gy/min at 37°C [138]. While these conditions are optimal for the development of lipid peroxidation in model membranes [139], the antioxidant defenses of these cells may be sufficient to prevent significant lipid peroxidation from occurring [139,485].

The localization of repair enzymes and nucleases on the cell membrane [163,417,445] means that free radical damage to membranes could interfere with repair and provides a rationale for regarding membranes as important radiation targets. Bacq and Alexander [38] proposed that a significant component of radiation effects is attributable to cell membrane damage. However, current evidence concerning radiogenic membrane damage does not indicate that such damage is of major significance except at exposure levels above those where DNA lesions are critical. At very high exposures, early effects on membrane permeability, i.e. allowing entry of macromolecules into vascular space, can be life-threatening.

Enzymes: A report of Zajac et al. [491] showed decreased enzymatic activities in irradiated microsomes. As reviewed by Singh and Singh [415], some enzymes are resistant to radiation, some are inhibited, and some are stimulated. There is no clear evidence to date to suggest that enzyme inactivation is a critical aspect of radiation damage, although several reports suggest that nucleic acid polymerases may be important targets leading to cell death [429,430].

D. Effects of Ionizing Radiation at the Functional and Systemic Levels.

1. Cell Survival Curves and Models of Cellular Inactivation.

At sufficient dosage, ionizing radiation kills cells or prevents their proliferation. Much of the current theory concerning the mechanism of irradiation damage to biological tissues is based on observations of radiation-induced cell inactivation in mammalian-cell tissue cultures. Use of such systems enables the researcher to determine the proportion of cells killed under a given set of experimental conditions. From the slope and form of curves relating the proportion of cells surviving (plotted with a logarithmic scale) to the dose (linear scale), various inferences and hypotheses concerning the mechanism of radiation damage may be drawn.

One of the characteristics of such curves which is often (but not always) observed is a so-called "shoulder" - a rather abrupt steepening in the slope of the curve above some critical dose range. This shoulder (indicating existence of a "threshold" dose, below which the cells are more resistant to the lethal effects of radiation) must be accommodated by any hypothetical model of radiation-induced cell inactivation. Only two of these models are mentioned here.

Target Theory: This model of cell inactivation assumes that each effect of ionizing radiation stems from a specific critical "hit" of a particular target molecule. This theory of radiation action has been dominant for many years. For a cell survival curve without a shoulder, the logarithm of the level of inactivation is directly proportional to the dose. A curve with a shoulder is interpreted as indicating a system in which accumulation of multiple hits (a specific number per cell) is required before the final hit can produce a lethal effect. When the dose is increased sufficiently that the energy absorption events per cell exceed this number, the low-dose threshold is exceeded and the slope of the survival curve steepens.

Repair Theory: This alternative to the multi-hit target theory, as an explanation of cell survival curve shoulders, rests on the fact that cells have a limited capability to repair radiation damage. According to this theory, even a single radiation absorption event may be potentially lethal but if the lesion is repaired in time (for instance, before cell division is attempted), cell death will be avoided. Because of such repair processes, cell death as a result of radiation damage is prevented or the degree of

lethality diminished at lower dose rates. At higher dose rates (those above the shoulder) the limited repair capacity (or "Q factor") is saturated and the slope of the cell survival curve is therefore steeper. Cell survival curves with no shoulder indicate that the cells have no repair capability for the damage being inflicted under the particular observation conditions.

Several types of observations support this theory, including those of increased cell survival if a given dose is administered as successive fractions (allowing between-dose repair by a system that would be overloaded if the same total dose were absorbed without interruption). Support for the repair theory is based upon the fact that certain chemicals (e.g. caffeine, actinomycin D) which are known to inhibit DNA repair also diminish or eliminate cell survival curve shoulders and the fact that curves without shoulders are seen for certain mutant microorganisms which have repair deficiencies.

Definitive experiments which will support one model of cell inactivation while being incompatible with others apparently have not been devised. However, support for the repair theory seems to be steadily increasing while the target theory no longer meets with the general acceptance that it enjoyed for many years. It seems likely that the ultimate model of cell inactivation will include elements of both theories.

2. Effects on Cell Division and Differentiation.

Chromosomal aberrations: The earliest microscopically apparent effects of radiation on cells are chromosomal alterations (such as breaks, deletions, and translocations). These alterations become detectable as the mitotic apparatus condenses for cell division. The visible chromosomal alterations are manifestations of earlier physical and chemical alterations of DNA molecules. If these alterations are sufficiently severe, the cell will fail to complete the transition through mitosis. At lower levels of DNA alteration, non-lethal damage may take the form of transmissable chromosomal abnormalities (mutations), abnormal differentiation in embryonic cells (terata), or neoplastic transformation.

Mutation: Mutations arise when a radiation-induced change in the DNA of reproductive cells is non-lethal to the cell and can be reproduced and transmitted during mitosis. The mutation is normally heterozygous (since it is extremely unlikely that the same damage would occur to the two separated members of a chromosome pair) and is usually recessive. Such a mutation may become apparent only after many generations when two individuals bearing the

mutation happen to mate. Although mutations occur spontaneously, ionizing radiation increases the mutation frequency. It has been estimated that the amount of radiation needed to double the spontaneous human mutation rate is 50-250 rads [321].

Teratogenesis: Teratogenesis is one of the well-recognized effects of ionizing radiation [449]. The fetus and embryo are particularly vulnerable because dividing cells are more sensitive to radiation than are resting cells. Among dividing cells, both undifferentiated embryonic cells and differentiated cells are less sensitive than are those which are undergoing differentiation. Therefore, radiosensitivity varies continuously during embryonic and fetal development. The nature of a radiation-induced teratogenic effect will depend in part upon the organ systems undergoing development at the time of irradiation. In the mouse, for example, abdominal hernia can be produced by irradiation on the 4th to the 16th day, cleft palate on day 8, 10, or 11, skeletal abnormalities on the 9th to 16th day, skull defects on day 12, etc.

Carcinogenesis: A significant effect of ionizing radiation in moderate doses is carcinogenesis [72,447,449]. The relationship has been conclusively shown in humans and other animals. The major types of cancer induced by human whole body external radiation are breast, thyroid, and lung cancer, and leukemia. For equal doses, high-LET radiations such as neutrons are more carcinogenic than low-LET radiations such as X-rays. It is widely believed that the essential carcinogenic event involves alteration of cellular DNA although this event may not be manifested as cancer for decades.

3. Acute Radiation Syndromes.

This term is used to refer to the effects of high-dose, whole-body radiation. These effects can be subcategorized under 3 major headings: bone marrow, gastrointestinal, and central nervous system syndromes [361].

Bone Marrow Syndrome: The bone marrow syndrome occurs at the lowest dosage of the three syndromes (about 100 R with a fatality threshold at 200 R). This syndrome is characterized by lethal damage to bone marrow and lymphatic stem cells, with consequent depletion of blood cells and the alteration of immune function. If exposure is sufficiently severe, death from the bone marrow syndrome occurs at approximately 3 weeks to 2 months after the incidence of radiation.

Gastrointestinal Syndrome: The gastrointestinal syndrome appears at a dose of approximately 500 R with lethality at 1000 R. It is characterized by gastrointestinal symptoms and failure of the intestinal mucosa which, if sufficiently severe, will result in death within about 2 weeks.

CNS Syndrome: The central nervous system syndrome, with encephalitis, meningitis, and CNS edema, is apparent at 2000 R. At about 5000 R, lethality from these causes occurs within 2 days - before the otherwise fatal gastrointestinal and bone marrow syndromes are expressed.

4. Summary of Effects of Ionizing Radiation on Biological Systems.

Because normal cell function is more vulnerable to disruption by alterations in DNA molecules than by alterations in molecules of other types, damage to DNA is believed to be the most critical effect in cells exposed to radiation. Depending on the number of cells which are damaged and the intensity of the damage, the effects of whole-body irradiation of animals can range from minimal and repairable DNA alterations with no life-threatening effects, through apparent recovery of the organism (but with residual life-threatening effects such as mutation, teratogenesis, and carcinogenesis), to massive cell lethality resulting in acute illness and death of the organism.

E. Effects of Ultraviolet Radiation on Biological Systems.

On the electromagnetic spectrum, ultraviolet radiation (approximately 280 to 400 nm in wavelength) falls between the ionizing X- and γ-rays (less than 10 nm) and the non-ionizing microwaves and radiowaves (greater than approximately 0.3 cm). Although ultraviolet radiation is not usually included within the concept of "ionizing radiation," its effects on interaction with biological tissues (particularly in the near ultraviolet wavelengths below 320 nm) have much in common with those of X- and γ-rays and will be discussed with them in the following sections.

Biochemical effects of ultraviolet radiation, including changes in DNA, RNA, enzymes (which may be either activated or inactivated), and synthesis of macromolecules are reviewed by Parrish et al. [340] and by Hall and Mount [190]. As do X- and γ-radiation, near-ultraviolet radiation generates superoxide radicals in cell culture growth media, an increase in chromosome damage, inactivation of enzymes, oxidation of polyunsaturated lipids, and membrane damage. In cell culture experiments, these effects can be prevented by exogenous superoxide dismutase [77,148].

Ultraviolet radiation can be absorbed by a variety of endogenous and exogenous biological compounds. As with other types of radiation, this absorption can result in generation of highly reactive, relatively short-lived intermediates (free radicals and ions) which through further energy transfers can chemically modify nucleic acids and other biomolecules or structures. As pointed out in the review by Parrish et al. [340], it has been concluded that the primary event in lethal ultraviolet irradiation of bacteria is alteration of DNA. Ultraviolet inactivation spectra for a wide variety of cells correspond relatively closely to the absorption spectrum of DNA, with a peak near 260 nm.

The mimicry of X- and γ-ray effects by ultraviolet radiation is emphasized by Peak and Peak [344]. Among these effects are changes in soluble and membrane-bound enzymes and in RNA, with these effects of near-ultraviolet (but not those of far-ultraviolet) being potentiated by oxygen (as are those of ionizing radiaton). One of the most prominent effects is the induction in DNA of cyclobutane dimers at adjacent pyrimidine base sites. Peak and Peak reported that this induction was several orders of magnitude less efficient and the dimers were more efficiently repaired for near-ultraviolet (greater than 320 nm) than for far-ultraviolet (less than 320 nm) irradiation. Radioprotectants which were protective against X-rays also protected against near- but not against far-ultraviolet radiation. In these respects, the effects of near-ultraviolet light more closely resemble those of X-rays than they do those of far-ultraviolet radiation [344].

Carotenoids protect against the adverse effects of ultraviolet irradiation through their action in quenching radiation-induced singlet oxygen and oxy-radicals [254] (as reviewed by Urbach et al. [451]), while other chemicals may potentiate ultraviolet-induced carcinogenisis.

F. Effects of Non-Ionizing Radiation on Biological Systems.

1. Types of Non-Ionizing Radiation.

The term "non-ionizing radiation" refers to microwave and radiowave (MW and RF) frequencies on the electromagnetic spectrum. Although frequency demarcations between types of radiation on the spectrum are not precise nor standardized, "microwaves" can be considered to include those with frequencies of approximately 300 kHz to 300 MHz and "radiowaves" are a sector of the radiofrequency domain which includes all frequencies below 300 MHz [304].

Microwave and radiowave exposures to the general population are now pervasive as the result of the large numbers of radio, television, and radar transmitters in use and the numerous other electromagnetic fields that are generated in a technological society. Exposures are commonly measured as mW/sq cm of exposed surface.

2. Contrasts Between Effects of Ionizing and Non-ionizing Radiation.

The most biologically significant contrast between these two types of radiation is that identified by their characterizations, ionizing and nonionizing. Below the infrared frequencies, quantum energies are too low to induce electronic excitations but are sufficient for resonance absorption. Therefore, radiation in the MW/RF ranges does not transfer its energy to biological molecules by the formation of chemically reactive ion pairs and free radicals. Rather, most of the energy transfer is accomplished by simple thermal conversion. This difference in the mode of energy deposition in irradiated tissue results in profound qualitative differences in the types and significance of resultant injury to the tissue.

While it is clear that at the most common levels of exposure, the health effects of non-ionizing radiation are not comparable to those of ionizing radiation, in practical significance it is also true that they are less well understood. There is still a residuum of uncertainty concerning the fundamental question of whether there are any adverse health effects of microwave and radiofrequency radiation which are not due to simple heat injury resulting from the thermal conversion of the absorbed energy.

Biological effects of non-ionizing radiation have been reviewed [181]) under the auspices of the NATO Scientific Affairs Division. The collection reveals areas of agreement, disagreement, and uncertainty concerning interactions of microwave and radiofrequency radiation with tissue. There is general agreement that tissue absorption of non-ionizing radiant energy is predominantly mediated by water, with conversion of the absorbed radiant energy to heat. High intensity exposures lead to irreversible thermal injury.

There is less general agreement as to whether low-intensity MW/RF exposures can also lead to non-thermal reversible or irreversible effects. Kaiser [232] states that "... well documented biological effects exist which arise from an irradiation at very low intensities (e.g. below 10 mW/cm^2)

where thermal effects can be excluded" and cites several studies. Reference is made to a theoretical concept which assumes the existence of metastable oscillating systems (e.g., in membranes) which are self-sustained by metabolic energy in special active states and can be triggered to a biological response by non-thermal absorption of low-intensity MW/RF radiation. The paper by Grundler [185] reaffirms previously published observations of changes in yeast growth rate caused by low intensity MW radiation with the effects being dependent on frequency and not correlated with power density in the range of 6 to 34 mW cm^{-2}.

Berteaud [59] suggests that thermal-gradient-mediated phase transitions in membrane lipids may be a primary site of MW/RF tissue alterations, even when no thermal gradients are apparent to the investigator. In contrast, Michaelson [305] reviews reported biological effects of RF/MW energy and finds no adequately documented evidence of non-thermal responses.

In any case, it is clear that except for the straightforward thermal injury of very high intensity MW/RF radiation, the biological effects of non-ionizing radiation are uncertain and are not comparable to those of ionizing radiation.

There has been no suggestion that chemical protection from non-ionizing radiation injury might be feasible. Work concerning protection has instead focussed on shielding and other dose-attenuation strategies. Therefore, MW and RF radiation will not be considered further in this review.

II. Endogenous Factors which Influence Radiosensitivity.

A number of characteristics of cellular biochemistry and physiology interact to determine natural or inherent radiosensitivity of both cells and the higher level structures which they comprise. Since some exogenous chemical radioprotectors may exert their effect by altering the normal balance between these endogenous factors, it is instructive to begin with a brief discussion of these factors and their relative effects. Some of these factors may be exploited for therapeutic gain to either increase or decrease natural radiosensitivity whereas others are not amenable to manipulation.

A. Water.

Energy deposition within a biological sample is dependent on a number of physical factors (as discussed in §I.C.2) whereas damage to critical targets (§I.C.3) is influenced by the interaction of energy with the molecules which

comprise the cell matrix. The deposition of energy in the target molecules themselves has been referred to as direct action whereas deposition of energy in the surrounding medium, with subsequent transfer to the critical targets, is referred to as indirect action. Since 55-70% of the cell mass is water [134], and since the absorption of energy in a mixture is in direct proportion to the mass ratio of its constituents [241], the products of water radiolysis as denoted in equation 1 have been thought to figure heavily in radiation damage to cells.

(1) $H_2O \xrightarrow{h\nu} H^{\cdot} + {}^{\cdot}OH + e^-_{aq} + H_2 + H_2O_2 + H_3O^+$

The formation of these highly reactive species has been extensively studied [92,130,415]. The deposition of energy is not uniform in radiolyzed water [284,405]. Approximately 10^{-14} sec after a pulse of high energy radiation, fast electrons interact with water molecules to produce the primary species of water radiolysis, according to equations 2-4.

(2) $H_2O + e^-_{fast} \longrightarrow H_2O^+ + e^- + e^-_{fast}$ $t_{\frac{1}{2}} = 10^{-16}$sec [125]
(3) $H_2O^+ + H_2O \longrightarrow H_3O^+ + \cdot OH$ $t_{\frac{1}{2}} = 10^{-14}$sec [261]
(4) $e^- + \text{water} \longrightarrow e^-_{aq}$ $t_{\frac{1}{2}} = 10^{-10}$sec [105]

These primary products of water radiolysis are formed in small localized regions known as spurs, of approximately 2 nm radius. The e^-_{aq} is formed throughout the spur whereas the formation of H_3O^+ and $\cdot OH$ are located primarily in the core of the spur in a sphere of ≈ 0.75 nm radius. These primary species undergo radical reactions in the spurs between 10^{-14} to 10^{-12} seconds by reactions 5-8.

(5) $e^-_{aq} + H_3O^+ \longrightarrow H\cdot + H_2O$ $k = 2.4 \times 10^{10}$ $M^{-1}sec^{-1}$ [175]
(6) $e^-_{aq} + \cdot OH \longrightarrow OH^-$ $k = 3.0 \times 10^{10}$ $M^{-1}sec^{-1}$ [193]
(7) $e^-_{aq} + e^-_{aq} \longrightarrow H_2 + 2OH^-$ $k = 4.5 \times 10^{9}$ $M^{-1}sec^{-1}$ [175] [129]
(8) $\cdot OH + \cdot OH \longrightarrow H_2O_2$ $k = 5.0 \times 10^{9}$ $M^{-1}sec^{-1}$ [291]

As the spur enlarges and the radical products begin to diffuse out into the surrounding medium, they undergo reactions with other radicals, forming

radical and neutral molecular products (equations 9-14).

(9)	$e^-_{aq} + H\cdot + H^+ \longrightarrow H_2$	$k = 2.5 \times 10^{10}\ M^{-1}sec^{-1}$	[193]
(10)	$\cdot OH + H\cdot \longrightarrow H_2O$	$k = 7.0 \times 10^{9}\ M^{-1}sec^{-1}$	[441]
(11)	$e^-_{aq} + H_2O_2 \longrightarrow OH^- + \cdot OH$	$k = 1.2 \times 10^{10}\ M^{-1}sec^{-1}$	[193]
(12)	$H\cdot + H_2O_2 \longrightarrow H_2O + \cdot OH$	$k = 9.0 \times 10^{7}\ M^{-1}sec^{-1}$	[432]
(13)	$\cdot OH + H_2O_2 \longrightarrow H_2O + HO_2\cdot$	$k = 2.3 \times 10^{7}\ M^{-1}sec^{-1}$	[440]
(14)	$\cdot OH + H_2 \longrightarrow H_2O + H\cdot$	$k = 6.0 \times 10^{7}\ M^{-1}sec^{-1}$	[441]

Within 10^{-3}sec of the receipt of the radiation pulse, the reactions of the primary free radicals are complete and a homogeneous solution of the products of water radiolysis as shown in reaction 1 has been formed. The yield of these species per 100 eV of absorbed energy (the G-value) is approximately as is shown in reaction 15 [93].

(15) $4.2\ H_2O \xrightarrow{h\nu}$

$$2.7\ e^-_{aq} + 2.7\ H_3O^+_{aq} + 2.7\ OH\cdot + 0.6\ H\cdot + 0.45\ H_2 + 0.7 H_2O_2$$

Within a spur at $< 10^{-12}$ sec after radiation incidence, the concentration of $H\cdot$ and e^-_{aq} may reach 10 and 100 mM respectively [415], while in the core of the spur, $H\cdot$ and $OH\cdot$ may be formed at concentrations which approach 0.5 and 2 M respectively [130,227,228,405]. As the dimensions of the spur increase over time and the products of water radiolysis undergo their reactions, the concentrations of the reactive species decrease so that by 10^{-3}sec a homogeneous solution is formed with concentrations of radicals which are orders of magnitude lower than what is initially formed ($< 10^{-12}$sec) in the core of the spur on interaction of water radiolysis products with molecular oxygen.

The yield and nature of radical and molecular species formed on irradiation of water is influenced to a great degree by the presence of molecular oxygen (reviewed by [78]). Molecular oxygen has a high affinity for the reducing radicals $H^\cdot$ and e^-_{aq}, forming perhydroxyl

(16)	$H^\cdot + O_2 \longrightarrow HO_2^\cdot$	$k = 1.9 \times 10^{10}\ M^{-1}sec^{-1}$	[432]
(17)	$e^-_{aq} + O_2 \longrightarrow O_2^{\cdot-}$	$k = 2.0 \times 10^{10}\ M^{-1}sec^{-1}$	[194]

(equation 16) and superoxide anion radicals (equation 17), respectively. The

rates of these reactions are so high that in oxygen saturated water, few solutes can compete with oxygen. Thus by 10^{-8} sec after irradiation, the radiolysis products in oxygenated water include:

(18) $HO_2^{\cdot} + {}^{\cdot}OH + O_2^{\cdot-} + H_2 + H_2O_2$

Oxygen interacts with the hydroxyl radical only under conditions of extremely high pH; under these conditions the ozonide ion $O_3^{\cdot-}$ is formed.

The reactions between organic compounds and the primary products of water radiolysis have been studied extensively. Under anoxic conditions, reactions of the hydrated electron and the hydrogen atom will be important. Reactions between the hydrated electron (e^-_{aq}) and organic compounds are, by definition, electron transfer processes. Since one-electron transfer reactions with most organic compounds do not produce stable species, the primary reaction products undergo secondary reactions to produce stable products. The hydrated electron undergoes addition reactions with many organic compounds, depending on their electron affinity and redox potentials [27,193]. The hydrogen atom (H·) participates in addition reactions with sites of unsaturation in organic compounds, abstraction of hydrogen from organic compounds and charge transfer reactions with metal ions [26,28]. The hydroxyl radical similarly reacts by hydrogen abstraction, addition to sites of unsaturation and electron transfer [153]. The reactivities of the superoxide anion radical [399] and the perhydroxyl radical [64] have been reviewed. In each case, the reactions of transients formed from water (and oxygen, if present) involve a competition between reactions of the radical species with organic solutes (equation 19) and with water (equations 20a-c) and the ratio of these reactions will depend upon the strength of the carbon-hydrogen bond and the stability of the free radicals produced.

(19) $RH + e^-_{aq}$ or ·OH or H· $\longrightarrow$ radical products

(20a) $e^-_{aq} + H_2O \longrightarrow H^{\cdot} + OH^-$

(20b) $^{\cdot}OH + H_2O \longrightarrow H_2O + OH^{\cdot}$

(20c) $H^{\cdot} + H_2O \longrightarrow H_2 + {}^{\cdot}OH$

The relative importance of direct and indirect effects in the genesis of radiation damage has long been debated. Whereas these effects are conceptually distinct, the practical boundaries between them have become blurred as

more is learned about the radiation chemistry of water. The practical difference between direct interaction of a target molecule with photons or Compton electrons (e^-_{fast} in equation 2) and the presence of a target molecule very close to a water radiolysis spur having very high concentrations of e^-_{aq} and ·OH is not clear [425]. Furthermore, the direct effects of radiation do not always result in damage at the site of energy absorption, as direct effects may also involve energy transfer processes [75,198]. Nevertheless, water does contribute to the expression of radiation effects in some simple model systems [34]. However, water content is not a variable which is amenable to manipulation during the exposure of mammalian cells to radiation, so while water radiolysis must be taken into account when describing the mechanisms by which radiation effects are produced, we are not able to alter this variable to change the radiosensitivity in higher cellular systems.

B. The Oxygen Effect.

1. Definition and Historical Notes.

Few substances have a more pronounced effect on cellular response to radiation than oxygen. As defined in radiobiology, the oxygen effect refers to the increase in the sensitivity of cells to ionizing radiation as the concentration of oxygen is increased from zero to a fixed value [244]. This effect was first noted by Holthusen in 1921 in experiments with Ascaris eggs [208]. At the time, this effect was incorrectly ascribed to the non-dividing nature of these cells under anaerobic conditions. Some years later, Anderson and Turkowitz defined the absence of oxygen during irradiation as the primary factor, showing that yeast cells dividing anaerobically were similary protected during hypoxia relative to the aerobic state [29]. Studies in a variety of test systems noted the sensitivity to oxygen (reviewed by Patt [342]), but studies conducted by Mottram [318] and later by Gray and colleagues [14] quantified this relationship, noted its clinical relevance, and brought it to the attention of radiation therapists.

2. The Oxygen Enhancement Ratio.

Survival curves (plotting the logarithm of the surviving fraction of cells [ordinate] against radiation dose [abscissa]; see §I.D.1) for mammalian cells irradiated in the presence or absence of oxygen show the same shape. However, the dose of radiation required to achieve a given level of effect (to reduce cell survival to a given level) is greater in the absence of oxygen than in its presence. The ratio of the dose required to achieve a given

cell survival in the presence of oxygen to that dose required to achieve the same effect under anoxic conditions is referred to as the oxygen enhancement ratio (O.E.R.). Although there is some controversy about the magnitude of the oxygen effect at low radiation doses [276,381,382](cf[243,244]), the effects of oxygen are said to be "dose-multiplying", that is, independent of the dose of radiation and the survival level. The O.E.R. varies with the type of radiation (see §I.C.2) and decreases with increasing Linear Energy Transfer (§I.C.2)[46]. For sparsely ionizing radiations such as X- and γ-rays, the O.E.R. has a value of between 2.5 and 3. Dose multiplication by oxygen requires the replacement of the anaerobic dose term (D) in algebraic relationships describing the survival of cells by a multiple of D, without deviating from the fitted curve [14].

In studies of the effect of oxygen on the radiosensitivity of isolated macromolecules, a rather confusing picture emerges. The inactivation of enzymes such as ribonuclease [206], carboxypeptidases [112], trypsin [294], ferricytochrome c [47] and others (cited in [215]) shows a dependence on the presence of oxygen. Yet, oxygen has been reported to confer protection in such systems in certain cases [120]. For the inactivation of transforming DNA, similar discrepancies have been noted. Several authors report no effect of oxygen [114,215] while others report protection by oxygen [68,195]. In relating these studies to the enhancement of damage by oxygen in whole cells or tissues, one must recognize that in the absence of the normal cellular architecture and biochemistry, markedly different physicochemical processes may dominate (see §II.D).

3. Characteristics of Oxygen Enhancement.

a. Dependence on Oxygen Concentration.

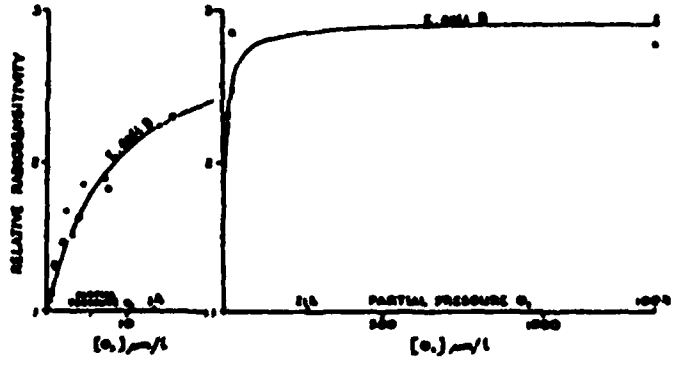
Soon after the presence of oxygen was recognized as an important factor influencing radiation sensitivity, the dependence of this effect on oxygen concentration was determined. Alper and Howard-Flanders [22] showed that for *E. coli* B irradiated in suspension, the sensitivity (S_p) of cells at oxygen partial pressure P relative to their sensitivity in anoxia (S_N) follows the relationship

$$\frac{S_p}{S_N} = \frac{mP + K}{P + K} \qquad (21)$$

where m and K are constants. The factor m represents the maximum sensitization, which may be interpreted as the oxygen enhancement ratio at high doses

of radiation. The constant K relates the rate of increase in sensitivity to the increase in the oxygen partial pressure.

Graphically, equation 21 describes a hyperbola, with radiation sensitivity increasing rapidly as oxygen concentration increases from zero to about 0.5 - 1% O_2 (Figure 1). Further increases up to the concentration of oxygen in air (21%) or to 100% serve to increase radiosensitivity only marginally. For the conditions under which equation 21 applies, m has a value of about three and K (interpreted as the partial pressure of oxygen at which the effect is half maximal ($K = \frac{m+1}{2}$) is about 5-10 μmol/l [22]. The experimental determination of the applicability of this equation is difficult [13] and is subject to the errors associated with the manipulation of low oxygen concentrations [100]. Perhaps for these reasons, although earlier studies had shown the general applicability of equation 21 to bacteria [22], yeast [211] and mammalian cells [122,109],



(Figure 1)

more recent research has described some deviation from this hyperbolic relationship. Millar et al. [309] have shown that by careful measurement of oxygen tensions in the range of 1-10 μM, a biphasic relationship is obtained between oxygen concentration and enhanced radiosensitivity of Chinese hamster V79-753B cells. This separation of the oxygen effect into components depending on oxygen concentration had been noted earlier in dry [358,359] and wet [152,436] bacterial spores and has been observed recently in yeast [314]. In an interesting re-analysis, Powers [357] has shown that even in data purporting to show a fit to the single component hyperbolic curve (equation 21) [274,483], transformations of the equation and a re-analysis of the data

according to a linearized relationship show two components to the oxygen effect. While non-linearity of this oxygen concentration-effect relationship may indicate multiple components which contribute to the oxygen effect, it is incorrect to attempt to deduce the parameters of these sub-components from a plot describing their combined effect [18].

b. Time Scale of the Oxygen Effect.

Investigations into the timescale in which oxygen acts to enhance radiation sensitivity can be categorized into two groups. Experiments on the pre-irradiation effect of oxygen, in which cells are allowed contact with oxygen for a fixed amount of time prior to irradiation, have provided evidence about the critical sites of oxygen-dependent radiation damage. Conversely, the admission of oxygen at fixed times post irradiation provides data on the lifetimes of that portion of anoxic radiation damage which can be altered by oxygen.

i. Pre-irradiation Oxygen Effects.

Howard-Flanders and Moore [213] studied the effect of transferring bacteria between gases of defined composition. Using an apparatus with a resolution time of 20 milliseconds (msec), they found that the bacillus Sigella flexneri exhibited a radiation response characteristic of the gas in which they were irradiated - irrespective of their incubation in a different gas more than 20 msec prior to irradiation. Thus, in bacteria, the radiosensitizing effect of oxygen is fully expressed within 20 msec. These results were confirmed by Shenoy et al. [408] using a rapid mixing technique for Serratia marcescens over a wide range of oxygen concentrations. However in experiments with mammalian cells in culture, two components to the oxygen effect were resolved in time [8,408]. At low concentrations of oxygen (1-10%), the cells required 40 msec contact with oxygen to achieve their full oxygen enhancement ratio. At higher concentrations of oxygen, the full O.E.R. was achieved at earlier times. These data were discussed in terms of several hypotheses, the most plausible of which is the existence of two sites within the cell at which oxygen may interact. Subsequent studies using a carefully designed technique of a similar concept [481] with another mammalian cell line have shown a similar timescale, though no evidence of time-resolvable components [482,483]. Thus, in mammalian cells, the effects of oxygen are expressed within several tens of msec after contact with the cells.

ii. Post-irradiation Oxygen Effects.

Oxygen added 20 msec after the anoxic irradiation of bacteria was without effect in altering the radiation response [213]. From this result, it was concluded that the reactive species, which are produced within these cells by radiation, decay in less than 20 msec to products which do not interact with oxygen. The refinement of fast response techniques has considerably shortened this estimate of the maximum lifetime of oxygen dependent damage. In fully hydrated bacterial spores, oxygen-dependent damage decays relatively slowly, following two parallel first-order reactions with half-lives of 9 and 120 sec [428]. In bacteria [149,478] and mammalian cells [273], the double pulse technique [150] yields an estimate of 0.1 msec for the maximum lifetime of oxygen-dependent damage. Conversely, the oxygen explosion method [302] consistently measures a half-life for decay of these species from 0.5 to 1 msec [301,303,477]. Although some disagreement has been noted, probably arising from the different techniques employed, it is clear that the transients produced upon irradiation which are induced in bacteria and mammalian cells under anoxic conditions are short lived, react rapidly with oxygen [303], and decay to products which do not interact with oxygen to increase radiation sensitivity.

4. Mechanisms of the Oxygen Effect.

The early theories of the mechanism by which oxygen increases the radiosensitivity of biological material involved the biochemical and physiological effects of oxygen which were known at the time [342]. However, as details of this sensitization were investigated, it became apparent that a physico-chemical mechanism of action was a more likely explanation for much of the oxygen effect. This view was championed by L.H. Gray and his colleagues whose arguments have been reviewed [356]. The temperature and concentration effects, the time of action of oxygen, and the variation of the oxygen effect with variations in radiation quality (LET) are all consistent with a physico-chemical mechanism.

The nature of this physico-chemical mechanism is the focus of the oxygen-fixation hypothesis [17,19,22]. In this theory, ionizing radiation is presumed to create free radicals within the target molecules of the cell which may undergo a number of reactions. If oxygen is present during or immediately after irradiation, this first reaction of the radiation-induced

radical (the "metionic" reaction [19]) will be to combine with oxygen, forming a species which prevents normal biologic functioning of the target (equation 22).

$$(22) \qquad R \xrightarrow{h\upsilon} R^{\cdot} \xrightarrow{O_2} ROO^{\cdot}$$

Since the lifetimes of most free radicals are short, oxygen must be present during or immediately after irradiation to be effective in fixing radiation damage; if oxygen is not present, the radical decays either to its original, biologically active form, or to an alternative damaged state [404]. To a first approximation, this theory fits some of the data recorded on the effect of oxygen in a wide variety of biological test systems. Thus, the requirement for oxygen to be present during or immediately after irradiation fits the observed lifetime of oxygen-dependent damage (§II.B.3.b). Also, the older measures of the dependence of radiosensitization on oxygen concentration described by equation 22 are consistent with one interpretation of that relation - the competition between oxygen fixation of radiation-induced radicals and their spontaneous decay or chemical repair by endogenous processes (§II.D).

Not all data fit such an idealized model of oxygen radiosensitization as the oxygen-fixation hypothesis. The effects of radiation on different endpoints of radiation damage (such as mutagenesis and the loss of reproductive capacity) were found to be differently modified by oxygen. In particular, the production of mutants by irradiation was found to be enhanced by oxygen to a lesser degree than the overall killing (reproductive sterilization) of the same bacterial strains [45,79]. Thus two types of damage were postulated [16]. One type, termed Type N, is relatively insensitive to oxygen and was identified tentatively as involving damage to the nucleic acid of the cell. The other component of damage, Type O, has a large oxygen enhancement ratio; no identification of the site of this damage was proposed. The experimental identification of multiple components to the oxygen effect (§II.B.3) presents the tempting possibility that Type N/Type O damage may have a physical explanation and may be amenable to direct observation. However, insufficient information is available on the relevant characteristics of these resolvable components to the oxygen effect to warrant speculation in this regard [14].

Whereas oxygen exerts much of its effect by interaction with radiation-induced free radicals, other effects of oxygen are also well known. Certainly the concentration of oxygen in the post-irradiation culture of cells affects their expression of radiation damage [109,110]. The influence of oxygen on the repair of radiobiological damage (widely interpreted as the recovery from sub-lethal or potentially lethal damage; §III.C.5) has received considerable attention [245]. Extremely hypoxic cells may be incapable of repairing sublethal or potentially lethal damage. This inhibition of repair probably results from metabolic deprivation and occurs at oxygen concentrations (< 50 ppm O_2) well below the radiobiological oxygen effect (< 1000 ppm O_2). Thus biochemical processes involving oxygen may also contribute to its radiobiological effects.

The significance of the mechanism of oxygen radiosensitization for chemical radioprotection lies in the interaction of the oxygen effect with other modifiers of cellular radiosensitivity. The pervasive distribution of oxygen and its profound influence on response to radiation sets practical limits to the maximum efficacy of some other modifiers, particularly in, but not limited to, those cases where the second modifier alters the oxygen effect. The elucidation of mechanisms of action of radioprotectors may be approached by studying their influence on the oxygen effect [357] or its components, in those test systems where oxygen has been shown to exert multiple effects.

C. Endogenous Radioprotective Substances.

1. Thiols.

a. Glutathione - Biosynthesis and Regulation.

Glutathione (L-γ-glutamyl-L-cysteinylglycine)(GSH) is a unique tripeptide that is characterized by a γ-glutamyl bond, which confers resistance to normal peptidase activity, and a thiol group, which is a good nucleophile, with a dissociation constant (pK_a) of 8.56 (for reviews see [53,184,295, 296,372,374,375] and [253]). At the normal redox status of cells, essentially all intracellular glutathione is present in the thiol form (reduced glutathione, GSH) with less than 5% present as glutathione disulfide (GSSG). The total cellular pool of glutathione also includes low molecular weight mixed disulfides, protein mixed disulfides and thioesters.

Extracellular space, including various body fluids such as bile, plasma and the glomerular filtrate, contains GSH, GSSG and mixed disulfides generally at very low (μM) concentrations [296,480]. These concentrations are two

or three orders of magnitude less than the glutathione concentration present in erythrocytes and body tissues in general. The glandular portions of the stomach and liver have the highest known concentrations of glutathione followed by the spleen, kidney, lung, and other organs and tissues [74,253]. The turnover of glutathione during normal physiological conditions is continuous with a wide variation of rates depending on the organ or tissue. The half-life of glutathione varies by tissue, being about one to three minutes in the plasma compartment, one hour in the kidney, two to three hours in the liver, and several days in the spleen, lung, nervous tissue and erythrocytes [253]. The liver is particularly susceptible to rapid and extensive depletion of glutathione by the formation of glutathione conjugates which are catalyzed by glutathione S-transferases.

Other thiols present in the liver include coenzyme A (1-2 mM in mitochondria; ~15 μM in cytosol [217,420]) and cysteine (0.1 to 0.4 mM); other thiols are thought to be of lesser concentration. The cysteine concentration is tightly controlled since a high concentration appears to be toxic to the cell. Cysteine dioxygenase is a highly inducible enzyme capable of rapid oxidation of cysteine to cysteine sulfinate which is rapidly converted to pyruvate and sulfite. Sulfite is then oxidized to sulfate within the intermembrane space of mitochondria by sulfite oxidase (for a review see [53]).

Depletion of liver glutathione is followed by a rapid resynthesis in which the cysteine pool turns over every two or three minutes. It has been firmly established that during a high rate of cysteine consumption, the liver utilizes the cystathionine pathway for its de novo synthesis of cysteine. In this pathway, the sulfur atom of methionine and the carbon skeleton of serine are utilized with the intermediate formation of cystathionine [376]. Exogenous sources of cysteine such as N-acetylcysteine and L-2-oxothiazolidine-4 carboxylate promote glutathione synthesis.

Whereas the regulation of the cystathionine pathway is not well understood, the rapid response to lowered glutathione levels is well demonstrated. Often, extensive resynthesis is accompanied by a so called "overshoot" in glutathione with a higher level after resynthesis than prior to depletion. This phenomena remains unexplained and potentially could be of value in maximizing cellular protection against the effects of ionizing radiation.

An extremely important aspect of glutathione is its interorgan status and function. Since it appears that the liver is largely responsible for

maintenance of the supply of plasma glutathione, and with the rapid turnover of plasma glutathione (1 to 3 minutes), the mechanism and regulation of the efflux of liver glutathione into this compartment is of considerable interest (for reviews see [53,295] and [184]). While much remains to be understood, it is clearly established that the efflux is almost exclusively as GSH and not GSSG. Some controversy exists concerning the relationship between the intracellular level of glutathione and the rate of its efflux [9,133].

The degradation of glutathione is initiated by γ-glutamyltransferase (EC 2.3.2.2)(5-glutamylpeptide:amino acid 5-glutamyltransferase) which catalyzes the removal of the γ-glutamyl moiety of glutathione (for a review see [295]). The enzyme can catalyze the transfer of the γ-glutamyl moiety to water or to certain γ-glutamyl acceptor amino acids and dipeptides if these acceptors are at millimolar concentrations. However, only under very specific conditions does it appear that this enzyme utilizes such amino acid substrates in place of water [295]. γ-Glutamyltransferase is located almost exclusively in the membranes of certain cells with its active site exposed to the extracellular space [448]. The kidney enzyme activity is localized in the brush border of the proximal tubular epithelium [413]. Only extracellular substrates containing the γ-glutamyl moiety appear susceptible to hydrolysis or transpeptidation. The rate of efflux of cellular glutathione, mainly from the liver, governs the rate of plasma glutathione degradation principally by the extracellular γ-glutamyltransferase of the kidney. Thus, under normal physiological conditions, the rate of turnover of liver glutathione is dependent almost exclusively on the rate of efflux of glutathione into the plasma compartment [299]. It has been estimated that about 80% of the total glutathione effluent from the liver is into this compartment while the remaining 20% is excreted into the bile [375]. Extracellular γ-glutamyltransferase in the biliary and intestinal epithelial cells degrade rapidly any GSH, GSSG, mixed disulfides or glutathione S-conjugates excreted via this route. However, millimolar concentrations of GSH and GSSG are present in the bile at the point of entry into the bile and nearly one half of such glutathione can be degraded in the bile duct under certain conditions [Reed and Ellis, unpublished data, 1982].

Recent evidence suggests that the kidney has the ability to alter the redox status of extracellular glutathione as well as all other thiols that have been examined. An early observation [25] on the oxidation of GSH to

GSSG found that this reaction was catalyzed by an enzyme in kidney homogenate which was distinct from γ-glutamyltransferase. Renal thiol oxidase activity has been characterized in detail and many of its properties established [328,329,331,334]. This oxidase is a component of the plasma membrane of the kidney tubular epithelium and catalyzes the oxidation of extracellular thiols only. Substrates include GSH, cysteine, N-acetylcysteine and dithiothreitol [328,329]. In contrast to γ-glutamyltransferase which is located in the brush border region, thiol oxidase is in the vasolateral part of the tubular epithelial plasma membrane. It has been concluded that its activity is probably restricted to thiols present in plasma [334].

Limited information exists on the uptake and reduction of low molecular-weight disulfides. It has been shown that freshly isolated renal cells are capable of the uptake and reduction of cystine to cysteine which probably occurs via a GSH-dependent sequence of reactions catalyzed by cystosolic thiol transferase and glutathione reductase [NAD(P)H](EC 1.6.4.2; NAD(P)H:oxidized glutathione oxidoreductase) [327,334]. Some evidence exists that renal cells [330] and lung utilize extracellular GSSG in a manner that does not involve extracellular degradation prior to cellular uptake.

Isolated cells appear to be useful in studying the metabolism of therapeutically important disulfide drugs. 2-Mercaptoethane sulfonate, a uroprotective agent [83] against oxazaphosphorine cytostatics, can be detected as the disulfide form in the plasma. However, the pharmacologically active form, the thiol, is excreted in the urine [334]. The disulfide form is readily taken up by isolated renal cells and reduced to the thiol [332, 334]. Freshly isolated heptocytes do not take up the disulfide form and do not accumulate either the thiol or the disulfide form. Freshly isolated hepatocytes also fail to take up appreciable amounts of cystine or to accumulate either cysteine or cystine [373]. These cells accumulate momentarily both cysteine and cystine in the presence of high concentrations of cysteine [52,53]. Hepatocytes isolated aseptically and placed in culture adapt within hours to greatly enhanced uptake and utilization of cystine [Klingensmith and Reed, unpublished data, 1984).

The failure of freshly isolated hepatocytes to take up and reduce low molecular weight thiols appears to be a failure of transport and not reduction. Evidence for this conclusion comes from cell-free experiments with liver and kidney homogenates that represent a reconstituted system containing

thiol transferase and glutathione reductase [NAD(P)H]. This system, under a N_2 gas atmosphere, readily converted 2-mercaptoethane sulfonate (disulfide form) to the thiol form by both liver and kidney preparations [334]. Therefore, a deficiency in disulfide uptake capability appears to exist with hepatocytes but not renal cells. It can be assumed that maintenance of the thiol/disulfide redox status in the plasma compartment is complex and related to several important processes. Initially the efflux of GSH from the liver provides the redox potential to convert cystine (which is the predominant form of the total cysteine/cystine in plasma) to cysteine and the mixed disulfide of cysteine and glutathione [373]. Cysteine and, in turn any remaining GSH, can convert other disulfides to mixed disulfides and their respective thiol forms. Utilization of disulfide and mixed disulfide by the kidney also contributes to an overall decrease of disulfide and mixed disulfide in plasma. Other cell types will also cause a decrease by uptake, but again, variations in ability for uptake and possible limitation in rate of reduction of mixed disulfide is observed [84]. In vivo oxidation of plasma thiols by renal thiol oxidase to disulfides remains to be demonstrated at the presumed concentrations of thiols in plasma because of the low affinity of this enzyme and the resulting high K_m value [328,329].

In mammals, GSH appears to be an important physiological reservoir for cysteine. The rapid response of the cystathionine pathway for converting methionine sulfur and serine carbon atoms to cysteine appears limited largely to the liver.

The half-life of GSH in the blood is very short (1-3 minutes) because of the high degree of activity of extracellular enzymes in the the kidney. These enzymes function in the degradation of GSH, GSSG and related mixed disulfides of GSH (for a review see [184]). Extracellular release of cysteine and cystine (from GSH and GSSG, respectively) is initiated by γ-glutamyltransferase followed by the action of one or more dipeptidases located primarily extracellularly in the kidney and intestines. This process may be essential to provide the cysteine and cystine that certain cell types require for growth and maintenance of intracellular GSH. A constant efflux of GSH from the liver, coupled with these extrahepatic enzymes functions to provide a constant cysteine supply; a portion of this released cysteine and cystine returns to the liver to help maintain the liver GSH content.

Separation of the mitochondrial and cytoplasmic compartments in isolated hepatocytes by digitonin disruption has led to the demonstration of the metabolic independence of the mitochondrial pool of GSH from that of the cytoplasm [299]. Hepatocytes subjected to acute oxidative challenge have shown a greater correlation of cell viability with the mitochondrial GSH pool than with that in the cytoplasm (for a review see [374]). Such depletion is without appreciable loss of cell viability unless accompanied by the additional stress that can occur in the presence of many drugs [374].

Reduced oxygen species ($O_2^{\cdot-}$, $^{\cdot}OH$, H_2O_2) may be derived from the consequences of ionizing radiation, the redox cycling of chemical agents, or endogenous production during normal metabolism. The deleterious effects of these species are collectively known as oxidative stress and may lead to cell death. Oxidative stress is known to be limited by the glutathione redox cycle. Metabolism of cytosolic and mitochondrial hydrogen peroxide by glutathione peroxidase is firmly established (for a review see [235]). GSH provides the required reducing equivalents, and glutathione reductase utilizes NAD(P)H reducing equivalents to maintain a continuous redox pathway to limit the hydrogen peroxide concentrations to steady state micromolar levels. There is scant evidence to establish the rate limiting step during a high level of oxidative stress.

b. Thiols and Free Radical Reactions.

As we have discussed in previous sections, free radical reactions play a major role in radiation-induced cellular damage. Consequently, many schemes for the protection of cell constituents from radiation damage involve protection by free radical mediated processes. Relevant to this topic, we briefly review the large body of literature on the types of free radicals formed from glutathione and other thiols, their mechanisms of formation and their subsequent reactions. Finally, theories of thiol-assisted radioprotection by free radical processes are presented as a stimulus to further experiments in this research area.

1. Formation of Sulfur-centered Radicals.

The most prominent sulfur-centered radical is $RS^{\cdot}$, the thiyl radical. Other sulfur-centered radical species include perthiyl radicals ($RSS^{\cdot}$), the radical anion $(RSSR)^{-\cdot}$, and the radical cations $R_2S^{+\cdot}$, $(R_2S)_2^{+\cdot}$, and $(RSSR)^{+\cdot}$. The production and disposition of all these radical

species is a complex process involving reactions which occur at rates close to their diffusion-controlled limit. Whereas much evidence has accumulated indicating that thiyl radicals are formed as transient species in cellular systems, the identification and biological role for these other radical species is not as well understood.

Thiyl radicals may be produced by hydrogen atom abstraction from the corresponding thiol either by carbon-centered radicals (equation 23) or by hydroxyl radicals (equation 24).

(23) $R^{\cdot} + R'SH \longrightarrow RH + R'S^{\cdot}$

(24) $^{\cdot}OH + RSH(RS^{-}) \longrightarrow RS^{\cdot} + H_2O\ (OH^{-})$

Furthermore, thiyl radicals may be formed upon reduction of transition metals by thiols (equation 25).

(25) $RSH + Cu^{2+} \longrightarrow RS^{\cdot} + Cu^{+} + H^{+}$

These three equations serve to place thiyl radicals at the center of molecular "repair" of direct radiation damage (equation 23), the scavenging of water radiolysis products in the indirect effects of radiation (equation 24) and the possible production of toxic products upon the autoxidation of thiols (equation 25).

Estimates of the rate of molecular "repair" of cellular targets by thiols have been obtained by measuring the rates of reaction of various thiols with model carbon-centered radicals. In solutions containing methanol and cysteamine, pulse radiolytic experiments show that the rate constant for equation 23 (in which $R^{\cdot} = {}^{\cdot}CH_2OH$ and $RH = CH_3OH$) is $k = 6.8 \times 10^7$ $M^{-1}\ s^{-1}$ [6]. This rate constant holds true (within an order of magnitude) for a variety of thiols and a small number of aliphatic carbon-centered radicals, measured with either the equilibrium

(26) $RS^{\cdot} + RS^{-} \rightleftharpoons (RSSR)^{-\cdot}$ ($\varepsilon_{410} = 9.4 \pm 0.5 \times 10^3\ M^{-1}\ cm^{-1}$)

at a pH close to the pK_a of the thiol [6,7] or with the probe 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS, $\varepsilon_{415} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$)

[484]. However, under the experimental conditions required to measure molecular repair by the reaction shown in equation 26, the extent of the repair reaction (equation 23) varies widely with even minor changes in the structure of the carbon-centered radical. Thus, Baker et al. [44] found (a) nearly 100% repair by the reaction shown in equation 23 to occur with glutathione and carbon-centered radicals from simple aliphatic alcohols, (b) lower repair for biological materials such as glucose and deoxyribose, and (c) no measurable repair for adenosine-5'-phosphate. With more complex target molecules, the carbon-centered radicals may undergo competing reactions [437], and the demonstration of molecular repair is constrained by the measurement techniques which have been employed. Perhaps with the probe ABTS [484], estimates of the molecular repair by thiols will be extended to more complex biomolecules [See §V.1].

Similar pulse radiolysis experiments have measured the rate constants of the reaction between thiols and the hydroxyl radical (equation 24). For RSH = glutathione, the rate constant for reaction 24 (k_{24}) is 1.3×10^{10} M^{-1} s^{-1} [367]; the formation of the thiyl radical from the reaction of a number of thiols with $^{\cdot}OH$ approximate this value [204]. However, thiols may differ in their contribution to molecular "repair" by their susceptibility to alteration at other sites in the molecule, as may be the case for glutathione [416]. This susceptibility may influence their capacity for radical scavenging.

Thiyl radicals may also be produced by the one-electron reduction of transition metals accompanying thiol oxidation to the disulfide [192] or to those oxygenated forms which identification has been hampered by instability. The autoxidation of cysteine has been reported to produce toxic metabolites, presumably reactive forms of oxygen [394], which may be responsible for the toxicity associated with the administration of cysteine [394,470].

Disulfides also produce thiyl radicals during irradiation through their interaction with organic carbon-centered radicals (equation 27), inorganic radicals (equation 28) and the hydroxyl radical (equation 29).

(27) $\quad R^{\cdot} + R'SSR' \longrightarrow RSR' + R'S^{\cdot}$

(28) $\quad {}^{\cdot}PO_3^{2-} + RSSR \longrightarrow RSPO_3^{2-} + RS^{\cdot}$

(29) $^{\cdot}OH + RSSR \longrightarrow RSOH + RS^{\cdot}$

It has been suggested that for disulfides such as cystine or penicillamine, reaction 30 may be quite important [33]. This

(30) $RSSR + {}^{\cdot}OH \longrightarrow RSS^{\cdot} + R(-H) + H_2O$

reaction certainly operates for bis(t-butyl) disulfide in which the t-butyl fragment is relatively stable [71] and contributes to the spectrum of products produced during the irradiation of cystine [363], penicillamine [364], and cystine-penicillamine mixed disulfide [365]. However, evidence from pulse radiolysis [367] and product analysis [336,337,338,363,364,365] experiments suggests that for many biologically relevant disulfides, reaction 29 predominates.

As mentioned above, the radical anion $(RSSR)^{-\cdot}$ is formed by the reversible equilibrium of equation 26. Presumably, this species may also arise from direct electron capture of e^{-}_{aq}, a fact which has been exploited in measuring the rate of decay of this ion in anaerobic experiments [367]. The radical cations formed from sulfides and disulfides [$(R_2S)^{+\cdot}$, $(R_2S.^{\cdot}.SR_2)^{+}$] and $(RSSR)^{+\cdot}$ have quite interesting properties [33], but the formation and structure of these species have unknown relevance to radiation biology.

ii. Reactions of Sulfur-centered Radicals.

By definition, the hydrogen donation (or electron transfer) from sulfur-containing species to carbon-centered target molecules (equation 23) may only be thought of as repair of radiation damage if the sulfur-centered radicals produced in the reaction are less damaging than the original target radical. Given the somewhat lower reactivity of sulfur-centered radicals compared with their carbon-centered congeners, this requirement may be met. However, sulfur-centered radicals still are very reactive, and may undergo a variety of reactions. Although these reactions (generally) serve to detoxify the radical from which they were produced, notable examples may be found of the production of toxic species involved in the disposition of sulfur-centered radicals.

The thiyl radical reacts in a variety of ways that may allow restoration of, or may destroy, the original state of the thiol. The thiyl radical

may dimerize with radical decomposition, forming the disulfide (equation 31). Alternatively, addition of molecular oxygen to the thiyl radical

(31) $RS^{\cdot} + RS^{\cdot} \longrightarrow RSSR$

(32) $RS^{\cdot} + O_2 \longrightarrow RSO_2^{\cdot}$

(equation 32) proceeds with a rate dependent on the structure of the thiol, being high for cysteine and glutathione (8 and 1.6 x 10^9 $M^{-1}sec^{-1}$, respectively) [48,367,400] and somewhat lower for simpler thiols (e.g., ethanethiol, penicillamine). Addition of oxygen to thiyl radicals results in products ($RSO_2\cdot$), a portion of which may react to regenerate the original thiol. One product of this oxidation is the sulfinic acid (G_{RSO_2H} = 1.8 for glutathione [260]) which may dimerize and subsequently react with a thiol to yield RSSR, O_2 and the sulfinic acid (equations 33, 34). Thus a disulfide is produced by the radiolysis of glutathione which may be reduced

(33) $RSO_2^{\cdot} + RSO_2^{\cdot} \longrightarrow RSO_2SR + O_2$

(34) $RSO_2SR + RSH \longrightarrow RSSR + RSO_2H$

to the thiol by the action of enzymes such as glutathione reductase in the presence of NAD(P)H. Other stable products of the radiolysis of glutathione at neutral pH include γ-glutamylserylglycine, GSO_2H, and the disulfide GSSG [260].

Thiyl radicals may also interact with certain biological materials which are classified as antioxidants, reducing agents, or electron acceptors. The electron transfer from α-tocopherol (vitamin E) or ascorbate (vitamin C) has been proposed to dispose of the thiyl radicals formed during molecular "repair" of cellular targets (equation 35 and 36)[484]. This reaction

(35) $GS\cdot + AH^- \longrightarrow GS^- + A^{-\cdot} + H^+ \quad k_{35} = 6 \times 10^8\ M^{-1}sec^{-1}$ [159]

(36) $GS^{\cdot} + \text{Vit E} \longrightarrow GS^- + \text{Vit E}\cdot$

follows the general order of radical reactivities (C > S > O) since it is likely that these products are initially oxygen-centered radicals which may subsequently form resonance-stabilized structures with delocalization of the electron density at the oxygen atom [292]. Subsequent electron transfer to

biologic acceptor molecules (§II.C.2,3,4) will detoxify the radicals and complete the cascade of electron transfer from ultimate acceptor to target radical (§II.C.1.c).

Whereas the sulfur-containing radical cations and anions may be of less radiobiologic importance, they undergo several interesting reactions. The radical anion $(RSSR)^{-\cdot}$ reacts with molecular oxygen to generate superoxide radical anion and the disulfide (equation 37)[97]. Also, the radical cation of

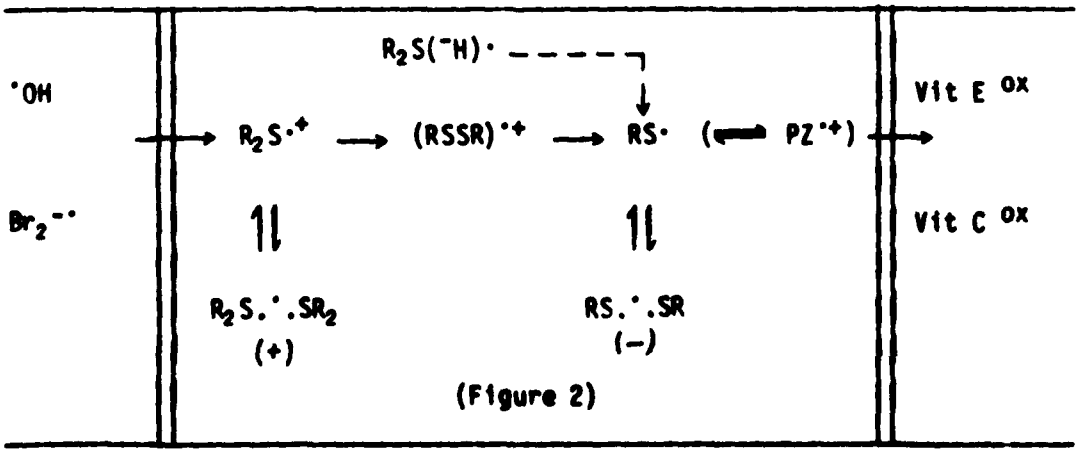
(37) $(RS\ .\cdot.\ SR)^- + O_2 \longrightarrow O_2^{-\cdot} + RSSR \quad k_{34} = 1.4 \times 10^9\ M^{-1}sec^{-1}$

(38) $CH_3\text{—}\overset{+}{S}\text{—}\dot{C}H_2CH_2CH(NH_3^+)COOH \longrightarrow CH_3\text{—}\overset{+}{S}\text{—}CH_2CH_2CHNH_3^+ + CO_2$

methionine is subject to an intramolecular reaction which results in decarboxylation and the formation of a carbon-centered radical [203] (equation 38). Thus not all sulfur-centered radicals are disposed without potentially harmful effects.

c. Overall Scheme of Sulfur-centered Radical Reactions.

The formation and disposition of sulfur-centered radicals can be summarized in several figures. Firstly, the chemical production and general order of reactivity of the sulfur-centered radical cations, anions and neutral species are shown in Figure 2. Highly oxidizing inorganic radicals such as $Br_2^{-\cdot}$ and the hydroxyl radical may cause the formation of



(Figure 2)

sulfide radical cations. Sulfide radical cations are more oxidizing than the disulfide radical cations which, in turn are more powerful oxidants than the thiyl radicals.

The reaction scheme from left to right is equivalent to electron transfer from right to left in the figure. In this figure, $PZ^{+\cdot}$ refers to the radicals produced upon oxidation of phenothiazine drugs. Several drugs of this class have been found to participate in radical reactions similar to those of the thiyl radicals [43]; their known antioxidant effects have been ascribed, at least in part, to their radical transfer properties [452].

A strong case is presently being made for thiol participation in coupling hydrogen donation reactions, as in the molecular "repair" of radiation damage, with electron transfer reactions leading ultimately to non-toxic biochemical reactions. The scheme of this coupling of hydrogen and electron transfer is shown in Figure 3 [484]. The initial step of molecular

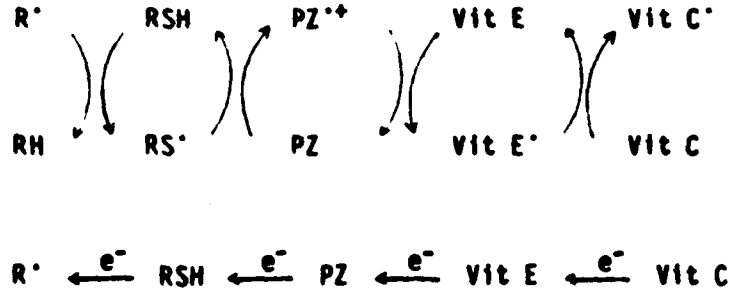


Figure 3

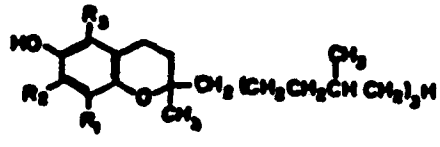
"repair" is hydrogen donation by a thiol; if this reaction occurs *in vivo*, RSH would be mainly glutathione. Except for thiyl radical formation, these reactions are envisioned as electron transfer reactions [160]. Obviously, in the absence of drug treatment, phenothiazine radical cations would not participate; recently the direct reaction of thiyl radicals with ascorbate anion has been observed [159]. The precise nature of the ultimate electron donor is speculative; reduced nicotinamide dinucleotide (NADH) and reduced cytochrome c may react at rapid rates with the glutathione thiyl radical [159].

Figure 3 presents an attractive picture for the disposition of radiation-induced free radicals. However, it rests on some limited data collected in highly purified model systems under controlled conditions. Several problems concerning the general applicability of this scheme must be resolved. First, the initial reaction certainly occurs for simple aliphatic carbon-centered radicals (§II.C.1.b). However when a variety of structures

are tested and the extent of reaction is determined, it has been noted that when the target radical is more biologically relevant, this reaction is quantitatively less important [44]. Furthermore, kinetic treatment of these data suggests that when competing reactions vie for the initially formed target radical, including intramolecular reactions, only a subset of the reaction products may be susceptible to this "repair" reaction [437]. Once thiyl radicals are formed from the target carbon-centered radicals, the extent of their participation (as outlined) in the electron transfer reactions outlined must be determined. As yet, no studies have been undertaken to address this point (See §V.5). Until these questions are resolved, the hypothesis for sequential free radical repair to progressively less noxious radical species, although conceptually (chemically) possible, must be considered somewhat (biologically) speculative.

2. Vitamins and Antioxidants.

Vitamin E is an efficient inhibitor of radical based reactions including lipid peroxidation in vivo (for a review see [439].)



$R_1 = R_2 = R_3 = CH_3$ α Tocopherol (α T):

$R_3 = CH_3$; $R_2 = H$ β-Tocopherol (β-T): R_1 =

$R_1 = R_2 = CH_3$; $R_3 = H$ γ Tocopherol (γ T):

$R_1 = CH_3$; $R_2 = R_3 = H$ δ Tocopherol (δ T):

(Vitamin E)

Autoxidation, in simple terms, is a chain reaction as described below in a reaction scheme of Burton and Ingold [90]. RH represents the organic substrate and initiation:

(39) Initiation: $RH \longrightarrow R\cdot$ or $ROO\cdot$

(40) Propagation: $R\cdot + O_2 \longrightarrow ROO\cdot$

(41) $ROO\cdot + HR' \longrightarrow ROOH + R'\cdot$

(42) $ROO\cdot + R'H \longrightarrow ROOR'^{\cdot}H$ ($\equiv R\cdot$)

(43) Termination: $ROO\cdot + ROO\cdot \longrightarrow$ non-radical products

$ROO\cdot$ the peroxy radical as the product.

Several individual tocopherols constitute vitamin E, and only recently has their relative and the absolute antioxidant effectiveness in vitro been clarified [90]. These chain-breaking phenolic antioxidants, ArOH, shorten the oxidation chain. Whereas chain termination by reaction 43 is suppressed, termination may occur by reactions 44 and 45 with n being the stoichiometric factor for the antioxidant.

(44) $ROO\cdot + ArOH \longrightarrow ROOH + ArO\cdot$

(45) $(n-1)\ ROO\cdot + ArO\cdot \longrightarrow$ nonradical products

In an inhibited reaction in which all $ArO\cdot$ are destroyed by reaction 45, the rate of autoxidation has been described by the following equation:

$$\frac{-d[O_2]}{dt} = \frac{k_3\,[RH]\,R_i}{nk_5\,[ArOH]}$$

where R_i is the rate of chain initiation.

The abstraction by peroxyl radicals of the phenolic hydrogens from these tocopherols has been described as the rate constant k_{45}. The values of k_{44} for α-, β-, γ-, and δ- tocopherols are 23.5, 16.6, 15.9 and 6.5 x 10^5 M^{-1} s^{-1}, respectively, at 30°C [90]. Each tocopherol was found to react with exactly two peroxyl radicals and all the tocopherols appear to be exceptionally good chain-breaking antioxidants in vitro. Further, the data of Burton and Ingold [90] are in agreement with in vivo tests of the relative biological activities of these tocopherols; α-T >β-T >γ-T >δ-T [96].

Rate constants have been reported for the reaction of superoxide radicals with ferricytochrome c, $k = 2.6 \pm 0.2 \times 10^5\ M^{-1}\ s^{-1}$ at pH 9.0 and ascorbate, $k = 1.52 \pm 0.1 \times 10^5\ M^{-1}\ s^{-1}$ at pH 9.9 [65]. It has been suggested that the facile oxidation of ascorbate by $O_2^{\cdot-}$ probably occurs via hydrogen atom transfer [399].

In aqueous media, reduced paraquat (methyl viologen MV^+) combines with O_2 to give a stoichiometric yield of $O_2^{\cdot-}$. In an aprotic solvent such as

dimethylformamide, MV^+ and $O_2^{\cdot-}$ combine in a 1:1 stoichiometry to form irreversibly a peroxy zwitterion adduct [$MV^+ O_2^{\cdot-}$] with the -OO- group at the 2-position [320]. Rapid decomposition via ring rupture and oxidative reactions yield a multitude of products [320].

Interestingly, no evidence has been obtained to indicate that $O_2^{\cdot-}$ acts as an initiator of radical chain reactions [399]. The powerful nucleophilic properties of $O_2^{\cdot-}$ in aprotic solvents do not exist in aqueous media [399].

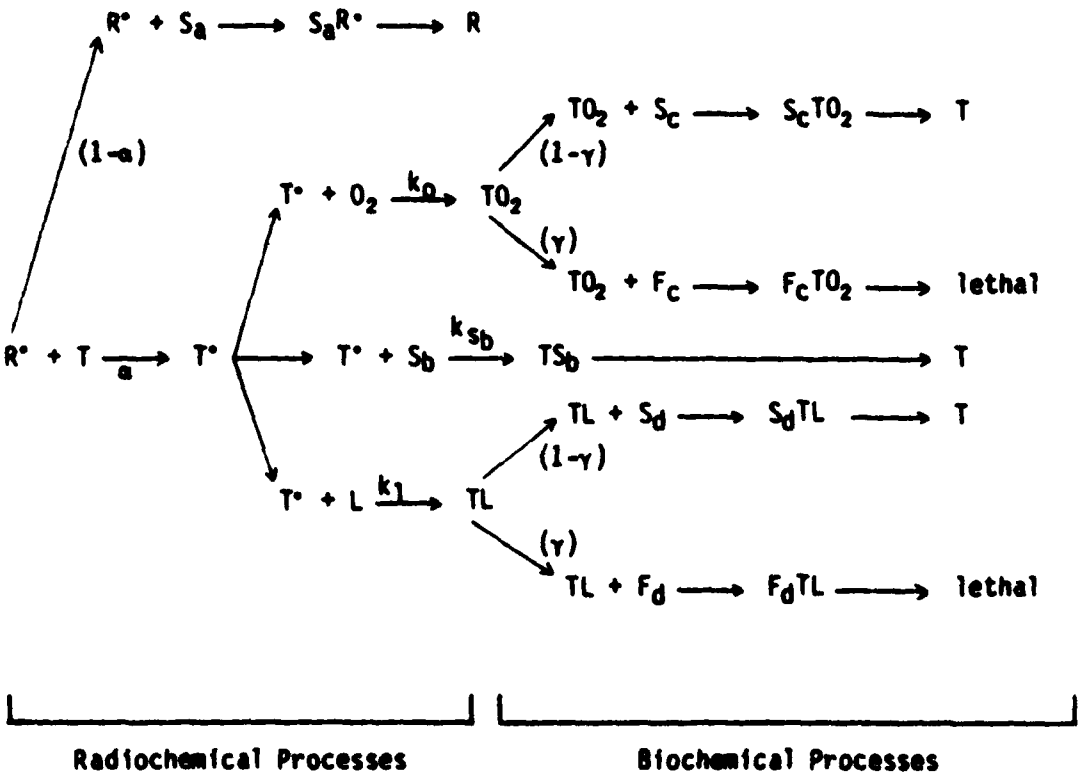
D. The Competition Model.

For nearly 30 years workers have extended the original observation of Alper and Howard-Flanders [22] on the role of oxygen in modifying the radiosensitivity of E. coli. Although molecular oxygen has the ability to increase the sensitivity of living cells to ionizing radiation more than any other agent, compounds containing the thiol moiety are by far the most active protective agents known. Thus, the quantitative relationship between ionizing radiation injury and oxygen supply continues to provide a useful means of describing the interactions of a variety of endogenous and exogenous agents that modify such injury. There is general agreement that the oxygen effect is mainly the reaction of oxygen with radiation induced free radicals at target sites which can result in permanent damage. Competition for these target site free radicals involves a large number of compounds with thiols having the greatest effectiveness.

Further understanding has led to the idea that an additional type of radiation damage occurs which is independent of oxygen [120]. Révész and coworkers [380] have extended the model to consider the initial reaction of radiation induced radicals with oxygen as being reversible and subject to biochemical alterations. The chemical events of ionizing radiation damage and biochemical processes are described in Figure 4 (1984, Lazlo Révész, personal communication).

Much of the recent work of Révész and coworkers involves the effects of ionizing radiation on glutathione deficient and proficient cells. The in vitro clonogenic survival of human fibroblasts with a genetically defined glutathione deficiency have been studied along with genetically related fibroblasts without glutathione deficiency [121]. The survival curves, obtained in an oxygen atmosphere, of both cell lines were similar but the

Figure 4. Schematic illustration of the radiochemical and biochemical processes postulated by the theory. $R^{\cdot}$ = unspecific radical; T = functional target; $T^{\cdot}$ = target radical; S_a, S_b, S_c and S_d = repairing species; O_2 = molecular oxygen; L = reactive species in absence of O_2; F = species which fixes damage irreversibly; α, β, γ = constants indicating proportions; k_O, k_{S_b} and k_1 = constants indicating reaction rates.



survival curves obtained in anoxic (argon) atmospheres indicated an oxygen enhancement ratio (OER) of about 1.5 for glutathione deficient cells. An OER of 2.9 was found when comparing the oxic and anoxic survival curves of the control cell line.

This evidence was used to support the theory of a competition model for oxygen and glutathione specifically since other thiols (cysteine and γ-glutamylcysteine) were present in the glutathione deficient cells [121]. Furthermore, these studies suggest that the major effect of exogenous thiols may be their scavenging action whereas endogenous glutathione exerts its major effect in the radical competition process with oxygen. Glutathione appears to be extremely efficient in the repair of radiation damage due to its high ability to elimiate free radicals by hydrogen donation. Révész [377] has proposed a qualitative difference between GSH^+ and GSH^- cells in their ability to repair oxically induced DNA damage even though no difference was observed in GSH^+ cells rejoining DNA single strand breaks (SSb) induced by hypoxia and radiation. However, GSH^- cells had a decreased capacity to repair oxically induced injuries which could indicate that the repair of SSb induced under oxic conditions requires GSH [377]. Exogenous thiols including GSH and dithiothreitol (DDT) could promote rejoining of SSb. On the other hand, GSH depletion with diethylmaleate does not change the rate of SSb repair [151]. The role of thiols in DNA repair is quite unclear and needs careful evaluation (See §III.C.5.b).

III. Chemical Radioprotection.

A. Physiological Resonses to Chemical Modulation of Cellular Radioprotection Systems.

Genetic and somatic alterations of cellular constituents occur during exposure to ionizing radiation. The extent of these alterations is related to the status of the cellular defense systems that protect cellular constituents and stabilize their functions. This section examines the general nature of radioprotectors and outlines the physiological responses involved in cellular radioprotection. Pertinent to "built-in" radioprotection at the cellular level during irradiation is the status of naturally occurring substances. Additionally, compartmentation of cellular protective systems must be considered since its role is beginning to be placed more in perspective relative to cell viability during chemical intoxication.

Cell viability and even cell survival is dependent upon the various inherent protective features. The protection of vital cellular constituents appears dependent on the structural integrity of the cell, the compartmentation of both functions and constituents, and the presence of certain enzymes. These enzymes include glutathione S-transferases, epoxide hydrolases, superoxide dismutases, catalase, glutathione peroxidases and glutathione reductase. Essential low molecular weight constituents include water, thiol compounds (particularly glutathione), vitamins C and E and the vitamin A precursor, β-carotene, although an absolute requirement for any one of these agents for cellular radioprotection has not been ascertained.

1. General Concepts.

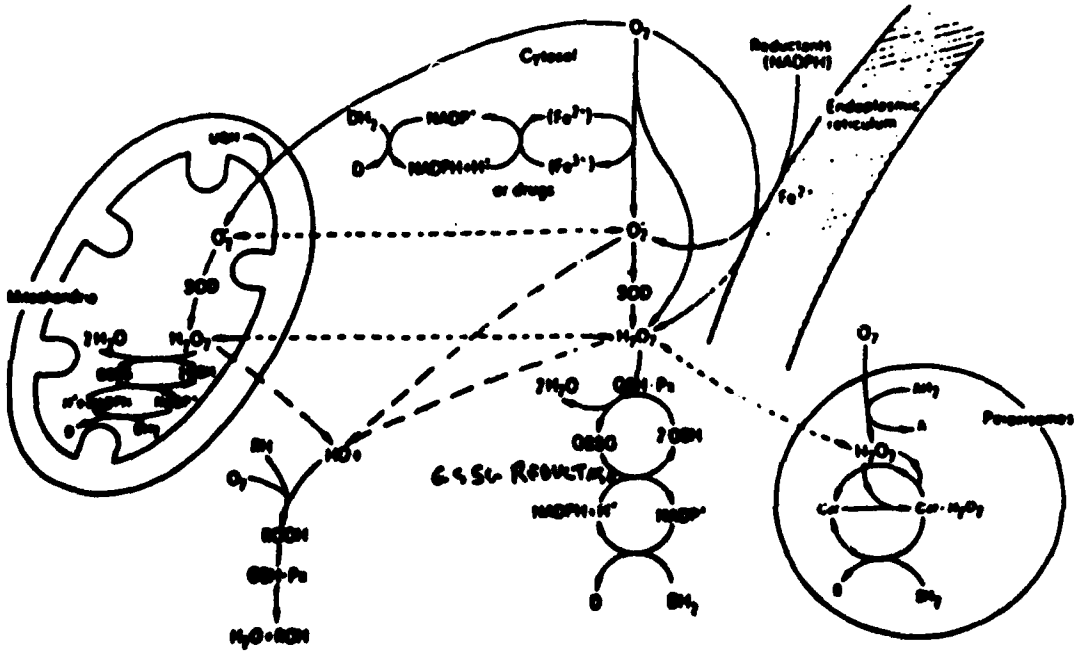
Cellular defense against injury can be defined in terms of the nature of the species causing the injury. In the case of ionizing radiation, at least a portion of that injury results from such highly reactive intermediates as the hydroxyl radical. These agents possess an inadequate electron density and attack cellular constituents at positions of higher electron density. The rates of such reactions are less dependent on the (relatively minor) variations in the inadequate electron density of the injurious species as they are on the electron density of the damaged cellular constituent. Reactions of this type are known to be susceptible to the presence of small amounts of agents that either liberate or scavenge radicals [433]. The importance of agents such as Fe^{2+} iron (for liberation) and vitamin E (for

scavenging of radicals), continues to be a subject of lively polemics among toxicologists.

Reduced species of dioxygen (including those that are free radicals) are endogenous to cells and tissues. However, ionizing radiation can result in a magnification of the total cellular content or the subcellular concentration of these reactive intermediates.

Cellular constituents that protect against free radical-mediated cellular damage reside in the subcellular compartments. These subcellular organelle compartments are important, but attention must also be paid to the distribution characteristics of both the prooxidants and antioxidants between the aqueous and lipid phases. Additionally, oxidation-reduction (redox) potentials vary with the degree of complexation with metal ions, macromolecules, etc. Thus the general nature of these reactions and even the protective systems are influenced by the particular milieu of the cellular interior.

Specific enzymes have a major responsibility for cellular protection against radical-mediated toxicity. The glutathione peroxidase/reductase redox cycle enzymes, catalase, and superoxide dismutases (SOD) catalyze reactions that are capable of realizing very rapid decreases in the concentration of reduced oxygen intermediates (Fig. 5).



(Figure 5)

Glutathione GSH) is a substrate for the glutathione peroxidase/reductase redox cycle (an enzymic reaction), but in non-enzymic reactions the cell primarily utilizes β-carotene, α-tocopherol, ascorbic acid and possibly uric acid [24] as important cellular protective agents.

2. Cellular Aspects of Radioprotection Systems.

a. Compartmentation.

The concentration and nature of nonprotein thiols and protein sulfhydryl groups has been a topic of long-standing interest. Vital cellular functions of protein sulfhydryls are numerous and cannot be enumerated here. However, the dynamic nature and relatively high concentration of protein sulfhydryl groups, which may exceed the concentration of total nonprotein thiols, (6-10 mM in liver) are closely related to the status and functions of nonprotein thiols. Protection of protein thiols from alterations (including the homeostasis oxidation state) involves the total cellular thiol:disulfide potential [494]. Subcellular distribution and concentrations of both non-protein thiols and protein sulfhydryls may relate closely to their respective functions. More than 90% of the total non-protein thiols present in cells appears to be glutathione. The presence of a discrete mitochondrial pool of glutathione was proposed by Vignais and Vignais [466]. Mitochondria retained GSH during experiments involving nonaqueous media [224,225]. Isolated rat mitochondria contain about 10% of the total hepatic glutathione with about 90% of it being present as reduced glutathione [225]. Wahllander et al. [472] reported the mitochondrial glutathione content to be 13% of total liver content by a nonaqueous extraction procedure.

Meredith and Reed [299] suggested that based on compartment water space [140,472], the mitochondrion maintains a higher glutathione concentration than the cytoplasm, 10 mM versus 7 mM, respectively. The apparent impermeability of the inner membrane of the mitochondrion led to the speculation that mitochondria maintain intramitochondrial glutathione by in situ synthesis [472]. Higashi et al. [199] have suggested that liver glutathione is a two-compartment physiological reservoir of L-cysteine. A labile compartment serves as a cysteine reservoir and as a more stable compartment, which is not readily available even during starvation. Cho et al. [102] have provided confirmatory evidence in that fasted and refed rats maintain a constant level of plasma cystine.

Studies on glutathione biosynthesis demonstrate separate pools of glutathione in the cytosol and the mitochondria with the in vivo turnover half-lives being 2 and 30 hr, respectively [299]. Short-term starvation depleted the cytosol pool but not the mitochondrial pool.

Differential depletion of cytosolic and mitochondrial glutathione of freshly isolated hepatocytes with glutathione depleting agents has permitted an evaluation of the protective role of intracellular glutathione. Chemical intoxication observed with ethacrynic acid [299], acetaminophen [298] or bromobenzene, suggests that short-term depletion of cytosolic glutathione does not cause a significant loss of cell viability. If depletion of cytosolic glutathione was accompanied by partial depletion of mitochondrial glutathione, then a rapid increase in loss of cell viability was observed. The effects of thiol depletion on cellular radiosensitivity have recently been investigated (e.g., [246]). Much more needs to be understood about the consequences of depletion of the pools of intracellular glutathione.

Hill and Burk [200] have speculated that vitamin E-deficient hepatocytes are more susceptible to oxidative stress than normal hepatocytes. Also, other oxidant defenses were unable to prevent lipid peroxidation that occurred under the incubation conditions employed in their experiments. Loss of cell viability during incubation was not accompanied by depletion of glutathione which indicates that some aspect of membrane fragility may relate to a specific function of vitamin E. Compartmentation may have an important role in these observations.

b. Redox Cycling.

A second role for glutathione is in a powerful radioprotection system against ionizing radiation damage which is mediated via the glutathione redox cycle. The cellular effects associated with reduced oxygen metabolism and subsequent redox cycling and lipid peroxidation have been reviewed by Kappus and Sies [235]. We are just beginning to understand and assess the energy required by the consumption of reducing equivalents that result from redox cycling.

The combined effects of protection by superoxide dismutase, glutathione peroxidase and glutathione reductase is:

(46) $4\ O_2^{-}\cdot + 4\ H^{+} \longrightarrow 2\ H_2O_2 + 2\ O_2$

(47) $2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$

(48) Net: $4\ O_2^{-}\cdot + 4\ H^{+} \longrightarrow 2\ H_2O + 3\ O_2$

Since superoxide anion radicals can migrate across artificial lipid bilayer membranes at temperatures above the lipid phase-transition [393], they may traverse membranes in general. However, their crossing membranes of erythrocytes [283] and granulocytes [170] is thought to occur via anion channels. This point could be important since most quinones are in membrane and the concentration of O_2 in the lipid plasma membrane is 8 times that in the aqueous medium [362]. Thus, superoxide anion radicals may be migrating in both directions across membranes from their site of formation. The rapid reaction of semiquinones with oxygen would indicate that semiquinones would not diffuse far in the presence of oxygen [360]. Protection by superoxide dismutase may indicate some superoxide anion production outside of cells [360].

Protection from reduced oxygen species therefore may occur in membranes by specific membrane-associated proteins which are capable of membrane protection and therefore can be shown to limit lipid peroxidation of membrane-associated polyunsaturated lipids.

An excellent example of the complexity of redox cycling has been discussed by Biaglow [61] concerning the reduction of nitro compounds that are radiosensitizers. Reduction of the nitro functional group to nitro radical anions is catalyzed by reductases including NADPH cytochrome P-450 reductase (AYH Lu, personal communication, 1982). The fate of such radicals depends on many factors including O_2 concentration and GSH. O_2 and GSH compete for the nitro radical anion electron and in turn form superoxide anion radical and glutathione thiyl radical, GS·, which then forms glutathione disulfide (GSSG). Decreasing the concentration of either O_2 or GSH appears to increase macromolecular damage. Such damage has been shown to be coincident with radiation damage and currently is being investigated extensively for therapeutic application in cancer treatment with nitro compounds such as misonidazole [89].

Protection against quinones appears to involve several aspects of cellular function involving oxygen reduction. Quinones can undergo either two-

electron reduction to corresponding hydroquinones or one-electron reduction to the corresponding semiquinone radicals [219]. However, the main cytotoxic effects of quinones are thought to be mediated through one-electron reduction to the semiquinone radical [35]. This radical is known to be capable of the formation of the superoxide anion radical by the one-electron reduction of molecular oxygen [165].

It has been suggested that, whereas the rate of NADPH formation is not limiting for monooxygenase activity, it may be rate-limiting for quinone-stimulated superoxide formation [360]. Simple quinones stimulate the formation of $O_2^{\cdot-}$ by isolated rat hepatocytes at rates up to 15 nmoles/min per 10^6 cells. Destruction of $O_2^{\cdot-}$ and water formation would require the consumption of 15 nmoles/min per 10^6 cells of intracellular GSH or nearly a complete turnover of GSH to GSSG and reduction back to GSH in two minutes. An equal quantity (7.5 nmoles) of NADPH must be furnished. However, Sies et al., [410,411] have calculated the maximum rate of NADPH production to be equivalent to 15 nmoles/min per 10^6 cells.

Sies et al., [411] have reviewed the metabolism of organic hydroperoxides and concluded that enzymatic reduction of organic hydroperoxides is the result of the activities of two GSH-requiring enzymes, glutathione peroxidase [EC 1.11.1.9] and glutathione transferase [EC 2.5.1.18]. Additional protection against hydroperoxides is afforded by endogenous "antioxidants" including α-tocopherol, ascorbic acid and β-carotene. Synthetic antioxidants such as butylhydroxytoluene (BHT) are thought to act primarily as mimics of α-tocopherol in the termination of the free radical reaction sequence.

The rate of cellular generation of NADPH from $NADP^+$ appears to be rate limiting for monooxygenase reactions. In intact liver, cytochrome P-450-dependent drug metabolism is decreased when an organic hydroperoxide is being reduced to the corresponding alcohol by glutathione peroxidase and, in turn, GSSG consumes NADPH-reducing equivalents in the conversion of newly generated GSSG to GSH [171].

A serious question has been raised concerning the role hydrogen peroxide may have in microsomal lipid peroxidation. Hydrogen peroxide reacts with reduced transition metals, especially ferrous iron, to generate the most oxidative oxygen species, the highly reactive hydroxyl radical, $\cdot OH$. This radical is thought to predominate in initiation of microsomal lipid peroxidation. However, Morehouse et al. [317] have concluded that hydroxyl radicals

generated from hydrogen peroxide do not initiate microsomal lipid peroxidation.

NADPH-dependent lipid peroxidation, which is initiated by NADPH-cytochrome P-450 reductase, may occur by hydroxyl radical formation through an iron-catalyzed, Haber-Weiss reaction [158]. Such lipid peroxidation may arise also from the formation of a reactive ADP-Fe-oxygen complex [442]. Possible events include the reduction of perferryl ion to the ferryl ion for initiation of lipid peroxidation. Superoxide anion may be the reductant:

(49) $$ADP\text{-}Fe^{3+} + O^{\cdot-}_2 \longrightarrow ADP\text{-}Fe^{2+}O_2$$

followed by

(50) $$ADP\text{-}Fe^{2+}O_2 \underset{+2\ H^+}{\overset{+2\ e^-}{\rightleftharpoons}} ADP\text{-}[FeO]^{2+} + H_2O$$

In contrast, NADPH-cytochrome P-450 reductase-dependent lipid peroxidation with EDTA-Fe may occur through a hydroxyl ion-dependent mechanism rather than an ADP ferrous ion-oxygen type of complex [88].

The effect of redox cycling can now be extended to calcium. Hydroperoxides can modulate the redox state of pyridine nucleotides and the calcium balance in rat liver mitochondria via the participation of the glutathione redox cycle involving glutathione peroxidase and glutathione reductase [280]. These workers have proposed that the redox state of mitochondrial pyridine nucleotides can be controlled in part by glutathione peroxidase and glutathione reductase and that oxygen metabolites are a factor in the balance of Ca^{2+} between mitochondria and extramitochondrial space.

A calcium-dependent process has been described as the common final pathway of toxic chemical cell death. Using primary cultures of adult rat hepatocytes, Schanne *et al.* [401] demonstrated that toxic cell death caused by ten different chemicals was dependent on extracellular Ca^{2+}. Similarly, Chenery *et al.* [101] showed that cultured hepatocytes exposed to CCl_4, and the calcium ionophore, A23187, were dependent on extracellular Ca^{2+} for the expression of toxicity. These investigators proposed a two-step mechanism for toxic cell death. The first step is the disruption of the integrity of the plasma membrane by widely differing mechanisms followed by a common final step: the influx of extracellular calcium across the damaged plasma membrane.

More recent experiments cast doubt on the commonality of this pathway for cell death. Recent studies by Acosta and Sorenson [2] have demonstrated that the toxicity of $CdCl_2$ is accelerated in cultured hepatocytes incubated with calcium-free media. These results are in direct conflict with those reported by Schanne et al. [401] and Chenery et al. [101]. In addition, Smith et al. [418] have demonstrated in freshly isolated hepatocytes that three different liver cell toxins, CCl_4, bromobenzene, and ethylmethane sulfonate (EMS), are far more toxic to hepatocytes in the absence of extracellular Ca^{2+} than in its presence. These studies suggest that certain aspects of toxic cell injury are not dependent on extracellular Ca^{2+}.

The reasons for variability in cellular responses to extracellular Ca^{2+} and chemical toxicants are presently unknown. One can only speculate that differences in media conditions play an important role. Reed and Fariss [374] have shown that the entry of extracellular calcium is not a prerequisite for cell death in isolated rat hepatocytes.

It is interesting to speculate that the susceptibility to cell injury afforded cells in calcium-free media may be the result of inadequate intracellular Ca^{2+} concentrations since A23187, permits the influx of extracellular calcium but does not cause cellular damage [374]. Indeed, these studies support the contention that the entry of extracellular calcium is not the cause of cell death but, more likely, the result of cell death.

A puzzling result of the extracellular Ca^{2+} studies is the accelerated toxic cell injury afforded hepatocytes incubated in calcium-free media. This phenomenon has been observed with numerous compounds including adriamycin + bis-(2-chloroethyl)-nitrosourea (ADR-BCNU), CCl_4, bromobenzene, and EMS which are depleters of glutathione [418]. Babson et al. [374] have demonstrated that ADR-BCNU toxicity is dependent on the level of intracellular glutathione. That is, cell death occurs once the level of intracellular GSH falls below 20% of its initial value. Accelerated cell death of hepatocytes treated with Ca^{2+}-free media resulted from an enhanced loss of intracellular glutathione [374]. This accelerated loss of glutathione has recently been shown to be the result of a rapid efflux of GSH which occurs regardless of the toxin used [374]. Consequently, the accelerated loss of intracellular glutathione in hepatocytes treated with Ca^{2+}-free media may explain the enhanced toxicity observed with ADR-BCNU or any other compound that relies on GSH depletion as its mechanism of toxicity.

Recent developments suggest that chemically induced cell injury results from changes in intracellular Ca^{2+} homeostasis as opposed to the influx of extracellular Ca^{2+}. These studies have demonstrated the depression of Ca^{2+} sequestration in liver microsomes and mitochondria after treatment with a variety of hepatotoxins. Furthermore, this loss in calcium retention appears to be related to alterations in the status of glutathione and reducing equivalents.

B. Theories of Radioprotection.

In the forty years since the research of the Manhattan Project suggested the possibility of chemical radioprotection, numerous theories have been proposed to account for the actions of radioprotectors. These theories range from molecular interactions between radioprotectors and the target molecules, which are damaged by ionizing radiation, to events at the organ level which influence the state of radiosensitivity of the organism. A theory of radioprotector action differs from the mechanism of action in that a single mechanism of action may be involved in several different theories by which radioprotectors exert their beneficial effects. This relationship should become clear in this section, in which we review the historical context of the theories of radioprotector action and explain which mechanisms may be involved in each theory. The individual mechanisms of action will be described more fully in the following section (§III.C.). Please keep in mind that several of these theories of radioprotection are mutually exclusive; however, in the absence of conclusive proof that they are <u>not</u> involved in chemical radioprotection we have chosen to present them in a historical context. This overview of theories is presented to orient the reader and to unify the mechanistic concepts to be presented in §III.C. in a field in which the lines between opposing theoretical frameworks have been quite heavily drawn.

1. Radical Scavenging.

The radical scavenging theory of radioprotection involves solely the indirect effect of radiation. The interaction of ionizing radiation with the solvent in biochemical systems, water, has been described in §II.A. The radicals produced from water radiolysis ($\cdot OH$, $\cdot H$, e_{aq}^-) exert damaging effects on critical constituents of model cellular systems and may be damaging in the whole organism, contributing to the sum of radiation damage.

Therefore, the removal of these water radiolysis products before they can interact with critical targets in cells should confer protection from radiation. This theory of radioprotection was first described when the free radical nature of radiation damage was first understood in the 1950's. It has survived to the present largely because at least a portion of the damage caused by ionizing radiation is likely the result of indirect action, and many very efficient radioprotectors are very good scavengers of water-derived radicals. The evidence for and against radical scavenging as a mechanism of radioprotector action is discussed in §III.C.3.a.

2. Hydrogen Donation to Target Radicals.

Both the direct and indirect effects of ionizing radiation produce free radicals in biological materials. Hydroxyl radicals, hydrogen atoms, and products derived from the aqueous electron, react with organic molecules to abstract hydrogen atoms from the molecule. Direct effects may also contribute to the formation of species in which a hydrogen atom is removed from the starting structure. In these circumstances, restitution of the original structure requires donation of a hydrogen atom. This hydrogen atom transfer has been observed between thiols and carbon-centered radicals (see §II.C.1.b.1.). In simple systems containing model carbon radicals (derived from aliphatic alcohols) and biological thiols (i.e. glutathione and cysteine), hydrogen atom donation with formation of thiyl radicals has been observed. However, these highly purified systems cannot be considered as directly comparable to those of cells or tissues, and the relevance of such reactions to biological target molecules is unknown. The literature on the pros and cons of hydrogen transfer as a mechanism of radioprotector action is discussed in §III.C.3.b.

3. Mixed Disulfide Formation.

The mixed disulfide theory was proposed by Eldjarn and Pihl in the mid-1950's and applies only to sulfur-containing radioprotectors which can form mixed disulfides with protein sulfhydryl groups. The effects of ionizing radiation may be expressed to a certain degree by the loss of biological function of critical protein molecules. These critical proteins may be protected from damage by the formation of temporary mixed disulfides in an exchange reaction which can be expressed as in equations 51 and 52. The formation of mixed disulfides

(51) Protein-SH + RSSR ⟶ Protein-SSR + RSH

(52) Protein-SS-Protein + 2 RSH ⟶ 2 Protein-SSR

serves to protect the labile sulfhydryl and disulfide groups on proteins from oxidative damage due to direct or indirect effects of radiation. The reversal of equations 51 and 52 would regenerate the original protein molecule after the ionization and free radical reactions initiated by radiation have been completed. In this way, enzyme active sites, the conformation of structural proteins, or critical ionophores may escape radiation damage at their thiol or disulfide moieties. Thus, the targets of radiation action are stabilized by mixed disulfide formation; since mixed disulfide formation is reversible, biologically-competent molecules may be reformed after the passage of radiation. The evidence for and against this mechanism of radioprotector action is discussed in §III.C.4.b.

4. **Release of Endogenous Radioprotectors.**

Révész and co-workers have proposed that exogenously administered radioprotective thiols and disulfides act by releasing endogenous radioprotectors which are responsible for the beneficial effects. This theory is restricted to exogenous radioprotectors having a thiol or reducible disulfide group, since the released endogenous radioprotectors (principally glutathione) are contained in naturally occurring mixed disulfide forms with cellular proteins. In a fashion similar to the mixed disulfide theory, the proponents of this theory have shown that the efficacy of radioprotection by exogenous agents correlates with their glutathione-releasing potential. The released glutathione is thought to prevent radiation damage by the other mechanisms of protection, i.e., by hydrogen transfer reactions, radical scavenging, etc. This theory is supported by the finding that glutathione may exert protective activity in ways that may not be mimicked by other thiol-containing radioprotectors [380]. However, more recent research on the nature of those thiols present in naturally occurring protein-thiol mixed disulfides tends not to support the theory, because only a small fraction of the low molecular weight thiols bound to protein have been identified as glutathione [81,278]. The evidence for and against this theory of radioprotection is discussed further under the title "enhanced protection against oxidative stress" in §III.C.3.c.

5. Biochemical Shock.

A theory of radioprotection, which was first proposed in the mid-1960's, and which has received little further direct experimental support. This theory was proposed by Bacq and associates [36,40,42] to overcome what they viewed as the inadequacies of the mixed disulfide and free radical scavenging theories of radioprotection. They proposed that radioprotectors (principally thiol radioprotectors) bind to cell membranes and induce the formation of lesions in the regulatory processes of the cell which leads to a radioresistant state. The hypothesis would explain the correlation of mixed disulfide formation with radioprotector efficacy since the formation of mixed disulfides was the first step in their proposed cascade of reactions that led to increased radioresistance. Consequently, no explicit mechanisms for the increased radioresistance were proposed at the time the hypothesis was formulated. However, the concept of "stress" reactions to a variety of environmental stimuli has recently been the subject of considerable investigation. Cells subjected to a brief hyperthermic treatment become more resistant to subsequent heat treatments (review [196]) and this thermotolerance is accompanied by specific biochemical changes. In the hour following heat shock, protein synthesis is virtually halted and, as translation function returns, the synthesis of a specific group of proteins is induced (Heat Shock Proteins, HSP) [226,236]. The synthesis of these proteins appears to be correlated with the development of thermotolerance [263,270]. These proteins are also regulated by environmental conditions other than heat shock [233, 264,479] and may be induced by chemical agents such as sodium arsenite or ethanol [269]. Concomittant with the development of thermotolerance, the yeast Saccharomyces cerevisiae acquires a resistance to radiation damage possibly by the induction of its DNA repair capacity [313]. The mechanisms by which thermal and radiation tolerance is increased by heat or these chemical agents is unknown. Recent work shows that altered glutathione metabolism is associated with the early stages of this effect [312,315]. Thus it is tempting to speculate that exogenously supplied thiols may be interacting with this stress response in some way - either by triggering induction (although in certain cell lines the response requires 8 - 10 hours to achieve its maximal effect) or by mimicing step(s) further down the pathway toward increased resistance. Paradoxically, other experiments have shown that exogenous thiols sensitize cells to thermal stress. It should be noted that

these studies [234,315] were all done in the presence of the exogenous thiol, rather than by treating the cells and washing out the thiol prior to the second application of heat stress. As heat may increase the autoxidation of exogenous thiols, this sensitization may be explained by invoking a heat-induced increase in the toxicity of the exogenous thiol. However, thiols may exert effects on cell constituents which result in observable increases in radiation resistance after they are washed out of cells [14,15,463]. Indeed, a recent report shows that the brief oxidation of GSH with diamide results in brief thermosensitization followed by the development of sustained thermotolerance [197]. Thus the induction of a stress response leading to radiation resistance by thiols or other radioprotection compounds needs to be investigated (see §V).

6. Hypoxia.

One of the major factors determining the radiosensitivity of a tissue is the degree of oxygenation during irradiation. As discussed in §II.B., oxygen contributes to the resulting damage by interacting with free radicals produced in both target molecules and in the solvent; it thereby increases the radiation damage which is measured. Consequently, a drug or procedure which decreases the extent of oxygenation should exhibit a relative radioprotective effect when compared to the fully oxygenated situation. When the oxygen effect was first recognized as an important factor in radiobiology in the 1950's, the concept was immediately applied to explain the radioprotective effect of a variety of radioprotectors. Two means by which hypoxia may be produced in tissues in vivo are by (1) reducing the delivery of oxygen to tissues either by altering oxygen transport or by redirecting blood flow to specific organs or portions of organs, or (2) by consuming the oxygen which is delivered to tissues in chemical or biochemical reactions, thereby limiting the oxygen available for participation in free radical reactions. Several hormonal agents may exert at least a portion of their radioprotective effect by altering blood flow in irradiated organs, thereby causing hypoxia. Also, at first, the radioprotection shown by sulfhydryl compounds was explained by the known consumption of oxygen during thiol oxidation. While a portion of the radioprotective efficacy of thiols may be attributed to this mechanism, other factors must certainly also be considered. The evidence for hypoxia by either mechanism is considered in §III.C.2.

7. Hypothermia.

The effects of temperature change on biochemical processes are well known. Consequently, temperature is a variable which has been employed to alter the radiosensitivity of cells and tissues. Hyperthermia is a promising adjuvant technique in cancer therapy, as it increases the susceptibility of cells to the toxic effects of chemotherapeutic drugs. Acute hyperthermia also increases the amount of damage produced by a given dose of ionizing radiation. However, as discussed above (§III.B.5. Biochemical Shock), the stress of hyperthermia is accompanied by adaptive processes which increase the resistance of cells to radiation. In contrast, the effects of suboptimal temperatures have been shown to result in a radioprotective effect [209]. Presumably, this protective effect may be explained by mechanisms which are different from the induced resistance resulting from prior hyperthermic exposure. The reduced metabolic activity accompanying hypothermia may allow more complete and efficent repair of radiation damage [239]. Alternatively, damage-producing reactions following the absorption of radiation energy may be slower or less complete and this effect may account for the reduced sensitivity [55]. Drug effects have only infrequently been explained by their action in causing hypothermia. A portion of the radioprotective activity of one drug, chlorpromazine, may be due to its ability to lower the body temperature; the protective effect and the profile of body temperature after chlorpromazine administration are coincident [60]. However, studies suggest that other drugs which produce hypothermia, including cysteamine, cysteine, serotonin, sodium fluoroacetate [39], cholinomimetics [424], and cyanide [458] may not produce the majority of their radioprotective effect by this mechanism [311].

C. Biochemical Mechanisms of Radioprotector Action.

1. Prevention or Reduction of Radiation Dose.[1]

a. Physical shielding of biological tissue.

b. Administration of blocking drugs or chelators.

2. Suppression of the Formation of Reactive Species.

When oxygen was recognized as an important factor governing the radiation sensitivity of biological materials, this concept was soon applied to the mechanism of chemical radioprotectors. Since the maximum dose-reduction factor which can be obtained for cells in culture or *in vivo* is approximately equal to the magnitude of the oxygen effect (§II.B), the reversal of the oxygen effect was a potential explanation of chemical radioprotection. Similarly, pharmacological agents known to alter hemodynamics *in vivo* were thought to be radioprotective by interfering with the delivery of oxygen to irradiated tissue [461]. It was shown early on that sulfhydryl radioprotectors incubated in closed vessels can rapidly deplete the solution of its oxygen content [183]. The evidence for these two mechanisms of action is reviewed below.

a. Cardiovascular Hypoxia.

The importance of a normally maintained blood flow and concommitant oxygen delivery mechanism in the maintenance of tissue radiosensitivity has long been appreciated [183]. Indeed, this topic has recently been the subject of investigation by radiation oncologists endeavoring to protect normal tissues within the radiation field from dose limiting side effects. These investigators have used physical limitations in blood perfusion [207] and more recently microsphere embolization of the intestine [279] or the kidney [161], perfusion with deoxygenated dextran-hemoglobin [202], and other techniques. While promising, these techniques are both very experimental and by definition will be limited to radiation therapy.

---

[1] These topics will not be considered at the specific request of the agency sponsoring this review. The topics are listed in this report only to indicate their logical place in the full spectrum of possible mechanisms of action of radioprotective drugs.

Alterations in tissue oxygen delivery by altering distribution of blood supply has also been proposed as a mechanism of action of chemical radioprotectors [461]. Compounds to which this mechanism has been applied include the biogenic amines (histamine, serotonin, norepinephrine and epinephrine) which exert specific pharmacological effects which were well recognized prior to the recognition of their anti-radiation potency. Also included in this group are thiols and disulfides, although the evidence for the production of hypoxia by this mechanism is slim. A third chemical which likely protects by inducing cardiovascular hypoxia is cyanide, operating by a vasomotor stimulatory mechanism [458].

Of the biogenic amines, the most widely studied compound in this group is serotonin, whose radioprotective effect was first described in 1952 [41, 182]. The early studies on the interrelationships between serotonin and radiation effects have been reviewed [423]. In the period 1952 to 1965, the determination of the mechanism of action of these substances was the focus of intense investigation; radiochemical (§III.C.3) and biochemical mechanisms were championed against the local hypoxia mechanism. In particular, evidence concerning the protection afforded chemical polymer model systems [11] was used to refute earlier claims that serotonin and other aromatic amines acted by local or systemic tissue hypoxia. In this experiment, the depolymerization of polymethacrylate is used as a model of radiation damage. Histamine, serotonin and epinephrine all exerted a "protective" effect in this system which cannot be due to their pharmacologic effect on oxygen delivery, since a non-biological system was employed.

However, since the biological radioprotective effect is the one whose mechanism we seek to explain, data from biological experiments should be given more weight. In this respect, the evidence for radioprotection as a property of the cardiovascular pharmacology of the biogenic amines is striking. Firstly, thymocytes irradiated in vitro are unprotected by histamine, epinephrine and β-phenylethylamine, at doses which offer good protection in vivo [454]. Correlations between the spleen oxygen tension and radioprotective efficacy are high for the drugs of this group and also help to explain differences in efficacy of histamine between strains of mice [460, 461]. Conversely, the oxygen tension in the spleens of mice and rats after injection of the thiols cysteine, cysteamine or aminoethyl isothiuronium (AET) shows a variable and inconsistent response, tending to increase, rather

than decrease the oxygen content [459]. Radioprotective tryptamines inhibit uptake of the dye neutral red into the spleen of mice, while non-protective congeners and amino thiols do not have this property [493]. Also, specific pharmacologic antagonists abolish the radioprotective effects of histamine, epinephrine, carbaminoylcholine [461] and serotonin [455,456]. If these drugs are acting to reduce the delivery of oxygen in radiosensitive tissues, then physically increasing the amount of oxygen available for delivery should reverse this effect. Indeed pure oxygen respired at pressures up to 60 p.s.i. reverses the radioprotective effect of serotonin, histamine, and epinephrine but causes much less reduction of cysteamine or cysteine radioprotection [457]. Finally, the state of intracellular oxygen tension in intestinal mucosa, estimated by measuring the oxidation state of the pyridine nucleotides $NAD(P)^+/NAD(P)H$, may be altered by serotonin, although these results were not clear cut [220].

Not all evidence favours hypoxia as the mechanism of radioprotection by the biogenic amines. As mentioned previously, indolealkylamines are protective in model polymer systems [11] and a recent report suggests that serotonin and 5-hydroxytryptophan protect mastocytoma P815A cells in vitro under conditions which preclude hypoxic radioprotection [398]. Protection of cells in vitro by β-adrenergic agonists may involve β-adrenergic receptors and the cyclic AMP system [419]. The data from the intracellular $NAD(P)H/NAD(P)^+$ reduction were equivocal, suggesting that intestinal radioprotection by serotonin may operate by a non-hypoxia mechanism. Also, inasmuch as most biological processes are interrelated, one can interpret serotonin or other biogenic amine radioprotection by other mechanisms. As examples, the enhancement of post-irradiation recovery/repair processes [32,57,180] (§III.C.5) or the effects of biogenic amines on endogenous thiols and their influence on radiosensitivity [179,310] (§III.B.4) have been proposed to account for biogenic amine radioprotection. However, the bulk of experimental evidence suggests that biogenic amines protect against ionizing radiation by affecting the cardiovascular system so that hypoxia is produced in tissues, exhibiting radiation damage.

b. Chemical/Biochemical Hypoxia.

An alternative means by which hypoxia may be induced in cells or tissues is by local consumption of oxygen by chemical or biochemical reactions. This mechanism of radioprotection is limited to the sulfhydryl (or

disulfide) containing radioprotectors which undergo oxidation reactions in which molecular oxygen is consumed. The chemical and biochemical oxidation of thiols has been reviewed [95,223] and efficient catalysts for the reaction between thiols and molecular oxygen have been identified. These catalysts include metal ions or their chelated forms in biological systems including haemin, cytochromes, and other metalloproteins. The metal ion catalyzed oxidation of thiols by molecular oxygen always follows the stoichiometry:

(53) $2RSH + O_2 \longrightarrow RSSR + H_2O_2$

(54) $2RSH + H_2O_2 \longrightarrow RSSR + 2H_2O$

(55) NET: $4RSH + O_2 \longrightarrow 2RSSR + 2H_2O$

For many years, the biochemical hypoxia mechanism was thought to be restricted by definition to thiols which can participate in reactions 53-54 either with or without catalysis [37,459]. As a result of drug disposition studies on cystamine (2-aminoethyldisulfide), in vivo reduction to the active thiol was recognized [142,464,465]. Furthermore, a "futile cycle" (Figure 6) has been proposed whereby there is oxidation of thiols by thiol-oxidizing enzymes (flavin-containing monooxygenase) and reduction of the resulting disulfides by the glutathione redox system [495], possibly with the assistance of thiol-transferase [288].

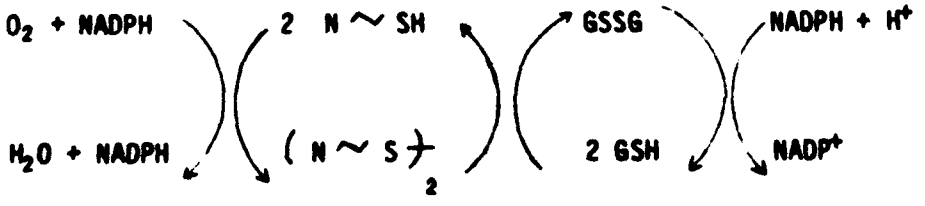


Figure 6: Redox cycle of aminothiol consumption of oxygen.

Evidence for and against this mechanism of radioprotection has been accumulating steadily since its suggestion by L.H. Gray [183]. Gray observed that unpurified cysteine (likely to contain traces of iron or copper) in buffer at pH > 6 rapidly removed oxygen from closed vessels. This effect was shown to operate in at least a part of the protective effect of cysteine. As

has been cautioned [14], reports on the importance of hypoxia in radioprotection from this period are difficult to interpret because the accurate measurement and control of low oxygen tensions in vitro were not often achieved [123]. Nevertheless, many reports were published either for or against the importance of hypoxia in sulfhydryl radioprotection. These early reports have been summarized [406,486]. Although most reports on sulfhydryl radioprotection acknowledge a contribution of protection by hypoxia, the extent to which this mechanism accounts for the sum of a radioprotector's actions remains controversial.

Early reports on hypoxia by sulfhydryl oxidation attributed the entire protective action of this class of radioprotectors to their tendency to produce this effect [183,205]. However, the effects of cysteamine, cystamine or cysteine are additive to the effects of severe hypoxia in vivo (breathing 5% oxygen) [123,391] although brief periods of respiring pure nitrogen produced effects which were not entirely additive [487].

Numerous investigations have shown (albeit reduced) radioprotection by sulfhydryl compounds under strict anoxia in vitro [99,210]. Measurement of oxygen content of tissues or blood in vivo show conflicting results: reports of a lowering of venous O_2-tension without change in the arterial O_2-tension after cysteine or cysteamine [389] conflict with reports of the variable or unchanged effect of thiols on tissue oxygen content [178,459]. As discussed in the previous section, the radioprotective effect of thiols was unchanged in animals breathing oxygen at high pressure [457]. If one accepts the reduction of pyridine nucleotide fluorescence as indicative of reduced intracellular oxygen tensions, sulfhydryl radioprotectors exert little influence on intracellular oxygen concentrations [220].

Recently, interest has again been focussed on thiol-induced hypoxia as an important factor in the radioprotection of mammalian cells by WR-2721 [S-2-(3-aminopropylamino)ethylphosphorothioic acid]. This compound is one of a group of compounds whose radioprotective actions are dependent on the hydrolysis of the material (in this case, a phosphoric ester) to liberate the thiol, which is the true radioprotective substance [248,250]. This agent has been investigated for clinical application because of its reported selectivity in protecting normal versus tumor cells [490]. The involvement of hypoxia in the mechanism of action of WR-2721 is important because one wants to selectively protect relatively well oxygenated normal tissue without

altering the already compromised radiosensitivity of relatively hypoxic (but not necessarily anoxic) tumor cells. Investigators at the Gray Laboratories have published an interesting series of reports [116,117,427] (also reviews [118,119]) whose collective impact is to question whether WR-2721 has any effect other than local tissue hypoxia. Experimental investigation on radioprotection in V79 spheroids, which are known to contain cells exposed to partial hypoxia, suggests that WR-2721 exerts both an oxygen-independent mechanism and an oxygen-dependent mechanism which is best expressed in these borderline-hypoxic cells [132]. Also Purdie et al. has shown that the product of WR-2721 dephosphorylation, WR-1065 [N-(2-mercaptoethyl)-1,3-diaminopropane] is capable of very rapidly depleting the oxygen content of suspensions of mammalian cells in culture [366]. The combination of oxygen identification as a critical factor in the clinical efficacy of WR-2721 coupled with experimental demonstration of its oxygen-depleting capability provides strong support for biochemical hypoxia as at least a large part of its mechanism of action.

All of these investigations are confounded by the lack of adequate ability to measure the oxygen concentration at the site of critical radiation effect, which likely is in or close to the nucleus. The pyridine nucleotide fluorescence measurements come the closest to achieving this objective, but these measurements reflect the oxygen tension in the sites of greatest flux of pyridine nucleotides, the mitochondria [220]. Furthermore, the observation of hypoxia or anoxia in closed vessels in vitro should be kept in perspective, as allowance must be made for factors in vivo which govern extracellular oxygen delivery and intracellular oxygen consumption.

3. Detoxication of Radiation-Induced Reactive Species.

When the physical interaction between ionizing radiation and the irradiated sample is complete (at $\approx 10^{-9}$ sec), radical species are present. Radical scavengers (reviewed in §a, below) may interact with water radicals to prevent damage to critical molecules in the cell which would otherwise have led to cell death. Radioprotectors may also react with organic or water radicals by hydrogen donation to "repair" these radical species (§b, below). Finally, since radiation damage approximates the "oxidative stress" which has been associated with active oxygen toxicity or the toxicity of compounds which undergo oxygen-dependent redox cycling, radioprotectors may support or

refurbish the natural cellular mechanisms of defense against oxidative stress (§c, below).

a. Free Radical Scavenging.

To the extent that radiation-induced radical formation occurs in the solvent (§II.A.), one mechanism of action of radioprotectors which must be considered is the removal of these products of water radiolysis before they are able to react with, and damage, the critical targets whose alteration leads to cell death. The extent to which the indirect (water radiolysis) effect is important in the expression of radiation-induced cell death remains a hotly debated topic among radiobiologists [349] cf. [12]. It seems reasonable to assume that products of water radiolysis do contribute to cell death and that the major damaging species is the hydroxyl radical (·OH) since the rates of reaction of various radioprotectors with ·OH correlate with the magnitude of their protective effect [107,390,396]. Although the involvement of the hydroxyl radical has generally been accepted, the assignment of the fraction of radiation damage to a specific percentage remains debatable [99].

A class of compounds whose protective action is generally accepted as involving radical scavenging is that of the aliphatic alcohols, including methanol, ethanol, ethylene glycol, and glycerol. A common feature of protection by these compounds is the large concentration required to achieve maximum radioprotection in vitro (1 - 3 M) [124,390] although by measuring parameters other than cell death, protective effects of radical scavengers may be noted at much lower concentrations [49,186]. Since their toxicity exceeds their radioprotective potency, the alcohols are not generally very effective in vivo. However, a compound whose maximum effect in vitro is similarly exerted at concentrations > 2 M is dimethyl sulfoxide (DMSO) [99]. DMSO is effective in vivo at a dose of 4.5 g kg^{-1}, which can be administered only because of the extremely low toxicity of the compound [30,31] As more is understood about the nature of the events interposed between free radical formation and cell death, the role of antioxidants has similarly been refined. Thus, classification of radical scavengers by the type(s) of radicals with which they interact is now possible, as is their protection against the initiation or sequelae of lipid peroxidation [370, 371].

Thiols may also participate in radical scavenging reactions, at rates which are close to the diffusion controlled limit (see §II.C.1.b.i). Even at these high rates of reaction, calculations show [349] that cysteamine (a very good scavenging thiol) would be required at a concentration of 30 mM to produce a dose reduction factor of only 1.3. Yet, cysteamine is effective to a greater degree at doses *in vivo* which would produce a concentration of 3 mM if the compound were distributed evenly throughout the body. This discrepancy is rationalized by suggesting that thiols are concentrated in the vicinity of critical radiation targets by electrostatic interaction or binding to the target molecule. This binding effectively raises their local concentration to a level at which their radical scavenging ability matches their protective potency (see §III.C.4.a.). On the other hand, thiols may also protect by mechanisms unrelated to radical scavenging.

One difficulty with the idea that radical scavenging plays a major role in radioprotection is the anomaly encountered with radiations of different biological effectiveness. As mentioned in §II.B.2, the oxygen enhancement ratio decreases with increasing linear energy transfer (LET). In concert with this observation, the yields of both $\cdot OH$ and e^-_{aq} decrease with increasing LET [258]. If the major mechanism by which radioprotectors act is to scavenge these radicals, and the contribution of these radicals to the total amount of radiation damage decreases, one would expect the effectiveness of radioprotectors to decrease in parallel with the radical yield. Glycerol protection of both bacteria and haploid yeast is relatively constant over a wide range of LET [23,289] and cysteine is equally effective in protecting against 5.2 and 27 MeV α-particles [20]. While it does not seem logical that a radioprotector should act by different mechanisms of action against the effects of radiation of different quality, these observations do not necessarily disprove the scavenging mechanism thought to operate with "soft" X- and γ-rays.

b. Hydrogen donation to target radicals.

A hypothesis of the mechanism of action of sulfhydryl radioprotectors, which has gained popularity in recent years, is the hydrogen donation hypothesis. This theory seeks to explain the radioprotection of this class of compounds by the reaction of thiols with carbon-centered radicals formed in critical target molecules (equation 23). In contrast to the radical

(23) $$R\cdot + R'SH \longrightarrow RH + R'S\cdot$$

scavenging hypothesis, this theory takes into consideration both the direct and indirect actions of radiation, i.e., target radicals may be produced by direct deposition of energy within the target or by the reaction of target molecules with the products of water radiolysis (primarily ·H and ·OH). The theory lies at the heart of the competition model of radiation protection (§II.D.) in that the molecular repair reaction shown in equation 23 is in competition with oxygen addition to the target radical (§II.B.4; equation 22).

Direct evidence for the validity of the hydrogen donation hypothesis is scant. Although the reaction shown in equation 23 has been observed [5,6,7, 159] (reviewed [484]), at present such observations can be made only indirectly in highly purified model systems. Thus, it is not possible to measure this reaction in whole cells, cell fractions, or even with biologically relevant molecules such as proteins or DNA. Furthermore, since this mechanism is limited to thiols, most of which are also efficient free radical scavengers, one has difficulty in distinguishing in biological systems between radical scavenging and hydrogen donation. Estimates of the contribution of hydrogen donation to radioprotection have been made in model systems in which indirect effects are diminished or eliminated (e.g. dry or frozen biological preparations). Under these conditions, sulfhydryl compounds are effective in protecting DNA [76] and enzymes [172]. Also, radical transfer between carbon-centered radicals in salmon sperm DNA irradiated at -196°C and cysteamine has been shown by electron spin resonance spectroscopy [326].

Based on the presumed competition between hydrogen donors and oxygen for the target radicals, one would expect that radioprotectors which are thought to act by hydrogen donation should be more effective when oxygen is excluded from the test system. This result was indeed observed for the protection by cysteamine [210] and mercaptoethanol [212] of bacteriophage DNA irradiated in aqueous suspension. However, in aqueous suspension, at least a portion of this protection may have been produced by the radical scavenging effects of these thiols, as the precautions against indirect effects may have been inadequate [14].

Difficulties with the hydrogen donation theory also hinge on the differential protection of hypoxic and well oxygenated cells. In contrast to results with bacteriophage, thiols are much more effective in protecting oxic than anoxic cell suspensions. This differential protection may be explained

either by invoking the theory of thiol oxidation resulting in oxygen consumption (which would tend to show a greater effect in well oxygenated cells [See §III.C.3.b]) or by the argument that hypoxic cells are already protected to their maximum degree by endogenous thiols and therefore derive little benefit from additional exogenous thiol. Furthermore, estimates of the rates of the reaction between model target molecules and either oxygen or thiols have shown that oxygen reacts with carbon-centered radicals as much as 200 times faster than the hydrogen-donating reaction [4]. Thus under physiological conditions and in the absence of any chemically induced hypoxia, the relative contribution of the hydrogen donating reaction is questionable. However, some experimental evidence does suggest that this reaction may account for a share of the somewhat modest radioprotective activity of thiols in the absence of oxygen.

c. Enhanced Protection from "Oxidative Stress".

The adverse effects of reduced oxygen species ($^{\cdot}OH$, $O_2^{\cdot-}$, H_2O_2) on cells or tissues are collectively known as "oxidative stress" (for overview, see [409]). Since these reactive compounds are produced during the irradiation of cells and tissues, one may refer to radiation as producing oxidative stress. Whereas acutely lethal effects of radiation may be mediated to a large degree by the indiscriminate and highly reactive hydroxyl radical ($^{\cdot}OH$) [107,396], other oxidants may also contribute to both acute and delayed effects. Perhaps as a result of the involvement in certain reactions of intermediary metabolism, cells have developed enzymes to provide protection against certain oxidants. Superoxide dismutase is effective in the destruction of the superoxide anion radical, converting two molecules of $O_2^{\cdot-}$ to one molecule each of O_2 and H_2O_2 [323]. Catalase [98] removes hydrogen peroxide, and glutathione peroxidase is a good catalyst for the detoxification of both hydrogen peroxide and lipid hydroperoxides [157]. Thus an intricate network of defenses has evolved to protect against oxidative stress.

Certain of these cellular defenses may be augmented by radioprotectors. We have previously noted the possible release of reserveirs of glutathione by exogenous thiol radioprotectors (§III.B.4); the released glutathione may function partly as the cofactor for either glutathione peroxidase or the

recently described glutathione-dependent labile cellular factor which protects against lipid peroxidation [188,201]. Alternatively, direct administration of the defensive enzyme may in some cases provide radioprotection. In vitro, added superoxide dismutase (SOD) is reported to protect erythrocytes [268] and DNA [492] from damage due to ^{60}Co γ-rays. SOD and catalase have been shown to protect certain enzyme activities associated with erythrocyte energy metabolism from γ-radiation in vitro [230]. Administration of exogenous SOD to mice [131] and rats [414] prolonged survival after lethal doses of radiation. In the most promising report to date, Petkau reported a dose reduction factor of 1.56 ± 0.04 after intravenous administration of SOD to mice [345]. This reduction of radiation lethality was attributed to protection of the proliferative capacity of bone marrow cells. On the other hand, radiation-induced inhibition of human lymphocyte blastogenesis was not protected by SOD or catalase, and indeed exogenous SOD and catalase depressed lymphocyte proliferation in vitro [242]. Other reports suggest that the protective effect of SOD may not be so striking [1,51]. In view of the many factors involved in the distribution and metabolism of enzymes administered in vivo [397], perhaps these conflicting reports should not be too surprising.

The possibility that chemical radioprotectors act by stimulating or inducing this enzyme seems even more remote. Indeed cysteamine in radioprotective doses is reported to inhibit activity of the enzyme in the bone marrow in vivo [255], and prior X-irradiation does not induce the activity of the enzyme in Drosophila [50]. Thus, the role of SOD in natural radioprotection and its possible therapeutic use as a radioprotective agent is unclear.

4. Target Stabilization.

Thus far, we have described means by which radioprotectors interact with the products of radiation to prevent or ameliorate radiation damage. Radioprotectors may also exert their action by interacting with the target for radiation damage. In this section we describe ways by which protective agents may stabilize the target to prevent or allow restitution of radiation damage.

a. Binding to DNA.

The idea that radioprotectors are effective by binding to DNA and stabilizing its structure was well summarized by Brown in 1967 [86]. This theory has been developed most extensively for the aminothiol radioprotectors, of

which cysteamine is the prototype. According to this theory, aminothiol radioprotectors bind to DNA and stabilize its secondary structure. This increased stability has several benefits.

With increased stability, the radioprotector-DNA complex is less susceptible to unwinding and subsequent loss of secondary structure that can occur when the DNA chain is broken by ionizing radiation or its products. Also, an increase in stability reduces the rate at which the DNA may be unwound for transcription or replication. In both of these cases, the increase in stability allows for a greater possibility that lesions produced in the DNA molecule can be repaired.

Evidence for the involvement of this theory in the mechanism of radioprotector action is mostly circumstantial. Cystamine, the disulfide form of cysteamine, was shown by Jellum in 1965 [221,222] to bind to DNA and the mode of binding has recently been shown to involve electrostatic forces [275]. The binding of cyst(e)amine[2] is almost entirely accounted for by binding to the sugar-phosphate backbone of DNA [82], interaction which involves binding between the cationic amino groups on the aminothiol or diaminodisulfide and the anionic phosphate groups of DNA [462]. Upon binding, the melting point (T_M) of the nucleic acid-radioprotector complex is raised, relative to the T_M of the nucleic acid alone [287,387,462]. Protection of DNA by cyst(e)amine[2] was found to be complete at low doses (< 20 Gy) of γ-radiation and high concentrations of cyst(e)amine[2]; at higher radiation doses, increasing concentrations of cyst(e)amine[2] protected against the destabilizing effect of radiation [387].

The correlation between DNA binding and radioprotective activity is only partial. It appears that DNA binding is not the sole requisite function. In contrast to the cystamine, guanidoethyldisulfide (GED), glutathione disulfide (GSSG) series, where DNA binding paralleled radioprotective potency, other diamines, such as cadaverine or diguanidines such as pentamethylene diguanidine, show good DNA binding but no radioprotective activity [249]. This result was explained by the possibility of localized radical scavenging by

---

2 In much of this work, cysteamine was dissolved in water or buffer and the interaction with DNA was monitored over a finite time period. In view of the capacity of cysteamine to be oxidized to cystamine, some question remains as to which material actually was bound to the nucleic acid in these studies.

the disulfides; alternatively, the disulfides may be easily reducible and therefore the DNA may be more accessible once the repair is complete.

Finally, the DNA binding hypothesis serves to readily explain some observed structure-activity relationships among the aminothiols (See §VII.A). Generally speaking, the presence of a thiol group in a molecule is not sufficient to express radioprotective activity; an amino group is required for highest activity, and the amino group cannot be separated from the thiol by more than three carbon atoms [128,431]. Presuming that the aminothiol is oxidized to the diaminodisulfide *in vivo* [494,495], an aminothiol having four carbon atoms between amino and sulfhydryl groups would result in a diaminodisulfide with ten atoms between the amino groups. In a study of DNA binding and stabilization by a series of α, ω-diamines having various carbon chain lengths, maximum stabilization was found with 1,5-diaminopentane and very low binding was observed with 1,10-diaminodecane [285,286]. Thus at the first inactive diaminodisulfide in the series, the relative binding is probably very low. However, this correlation does not hold up uniformly, since a series of N-(alkyl)-disulfides and thiosulfates were found non-concordant for DNA binding (measured using equilibrium dialysis) and radioprotective activity [164].

b. Mixed disulfide Formation.

This hypothesis, presented in the mid-1950's by Eldjarn and Pihl [141] involves the reversible formation of disulfide bonds between proteins and administered sulfur-containing radioprotectors. Following on the observation of mixed disulfide formation *in vitro*, the interconversion of cysteamine and cystamine *in vivo* was demonstrated. Eldjarn and Pihl also noted that after administering ^{35}S-labelled cysteamine or cystamine, most of the radioactivity was bound to protein in mixed disulfide linkage at the time of maximum protective activity [141]. Subsequently thiol/disulfide exchange reactions (equations 56 and 57) were documented, and the presence of substantial amounts

(56) $$RSH + R'SSR' \rightleftharpoons RSSR' + R'SH$$

(57) $$RSSR' + RSH \rightleftharpoons RSSR + R'SH$$

of mixed disulfide (RSSR') were found to be present in such a system at equilibrium [142,144]. A positive correlation between the rate and extent of

mixed disulfide formation and radioprotective activity was demonstrated for a variety of thiol compounds [143]. The regeneration of native protein may be accomplished by thiol-disulfide exchange (in which GSH functions as RSH in equation 57, possibly catalyzed by thiol transferase) and by subsequent action of the glutathione redox system coupled with glutathione reductase and NADPH [348].

Certain enzymes, especially those requiring an essential sulfhydryl group, were found to be protected from X-rays by forming mixed disulfides [350]. Other studies failed to show appreciable change in enzyme radioresistance upon mixed disulfide formation with a radioprotector [368]. The mechanisms behind enzyme protection were originally proposed to involve radiation-induced heterolytic cleavage of the disulfide bond of the mixed disulfide (equation 58). Such a reaction would theoretically yield low

(58) $\text{ProteinSSR} \xrightarrow{h\nu} \text{ProteinS}^- + {}^{\cdot}\text{SR}$

molecular weight thiyl radicals and protein thiols in one half of these radiation-initiated reactions [347]. However other experiments indicated that this heterolytic reaction may not occur [126], but rather that the mixed disulfide prevents interaction of the critical thiol with other radiation-damaged portions of the molecule [127]. The hypothesis of Pihl *et al.* suffers from several flaws. First, the measurement of equilibrium constants and rate constants has been criticized on the basis of the lack of consideration which was given to pH effects. Critics contend that inadequate attention was given to differences in the degree of ionization of the various radioprotectors when correlations were made between the equilibrium formation of mixed disulfides and radioprotector efficacy [176]. When one removes from consideration those candidate thiols which are widely different in degree of ionization and in steric hindrance to the reaction, little difference in rate or degree of mixed disulfide formation is observed. In defense of Pihl's correlations, it should be mentioned that all his measurements were made at 37°C and pH 7.4, conditions which are likely to approximate the situation *in vivo*. Thus candidate thiols, which do not exhibit ionization or steric factors under these conditions, are likely to exhibit similar behavior also *in vivo*, and so they should not be excluded from consideration *a priori*.

Perhaps the most serious fault with the mixed disulfide hypothesis is its failure to explain the radioprotection of nucleic acid, which is thought

by many to be the prime radiation target. Sulfhydryl groups are restricted to proteins, most of which are quite radioresistant relative to tissues, cells, or DNA *in vitro*. Identification of sulfhydryl-containing critical targets would support the hypothesis of protection by mixed disulfide formation. Some progress in this direction was made with the demonstration of X-ray inactivation of the initiation factor δ DNA-dependent RNA polymerase [429] and its protection by mixed disulfide formation [430]. However, the radiation-induced inactivation is produced at relatively high radiation doses, raising doubts about the relevance of this observation to the situation in cell inactivation studies. This type of experiment should be extended to other critical enzymes in DNA metabolism.

5. Enhancement or Protection of Recovery or Repair Processes.

After ionizing radiation has damaged cellular molecules, this damage may be expressed by the attendant biological consequences (cell death, mutagenesis, altered function) or it may be repaired, and the cell may recover from radiation-induced effects. For the purposes of this section, we have adapted the definitions of the terms "recovery" and "repair" as they are considered by Orr [333]. By these criteria, recovery [*i.e.* "Elkind recovery"; recovery from sublethal damage (SLD) or potentially lethal damage (PLD)"] refers to the whole cell and its response to radiation damage; recovery is measured as cell survival, death, or division time. On the other hand, repair ("DNA repair") refers to processes carried out on the molecular or sub-cellular organelle level. As pointed out by Orr, recovery and repair are related, though not necessarily always closely related.

The importance of recovery (and by inference, repair) lies in the end result desired. The observed decrease in radiotherapy efficacy with increasing dose fractionation [162] presents difficulties for cancer therapy presumably due to recovery from radiation damage in tumor cells; for increased radiotherapy efficacy, one would want to inhibit the processes governing recovery [231,319,438]. Conversely, for accidental radiation exposure, one would wish to enhance or stimulate this repair process to prevent radiation damage. The latter concept is the focus of our review.

a. Recovery from Radiation Damage.

Cellular recovery from radiation damage was described by Lea [266] in 1938, but the reports of Elkind and Sutton [145,146] stimulated interest in its effects and mechanism. Various metabolic factors are important in the

expression of recovery, including oxygen [87,277](for a review see [245]). The effects of radioprotective substances on the recovery process appear to have not been investigated extensively. Presumably thiols could be tested for their effect on the recovery process between doses of a split-dose experiment, provided they were removed from the medium prior to the second dose of radiation (without removal, the effect of radioprotective substances on the second dose of radiation might be misinterpreted as an effect on recovery).

The term potentially-lethal damage is used to denote circumstances under which, by appropriate treatment of irradiated cells, the expression of radiation lethality may be avoided. This term was coined by Phillips and Tolmach in 1966 [346] to describe their observation that cells treated immediately following irradiation with cycloheximide to inhibit cell division were more resistant to the radiation damage. Similar results have been observed for the holding of cells at temperatures suboptimal for growth [56]. While some work has been performed on the identification of substances which inhibit the recovery from potentially lethal damage, little investigation has been done on the stimulation of this biological phenomenon.

b. Repair of Radiation-damaged Structures.

Macromolecular structures damaged by radiation may be repaired by enzymes. DNA repair is a well-researched function of bacterial and mammalian cells [191], and this process has recently been the subject of numerous reports in radiation biology [21]. The involvement of ADP-ribosylation in repairing DNA strand breaks produced by radiation is under investigation, and the suggestion has been made that such repair of DNA strand breaks is necessary for cellular recovery from radiation damage [281]. Glutathione (GSH) may also be involved in the repair of DNA single-strand breaks (SSb) as has been suggested using cells genetically deficient in GSH synthesis [137,378] or in which GSH deficiency was produced by D,L-buthionine-S,R-sulfoximine (BSO) [378] or hypoxia/misonidazole treatment [135,379]. Interestingly, cells which are genetically deficient in glutathione synthetase [265] have increased intracellular amounts of γ-glutamylcysteine, cysteine, and other low molecular weight thiols [137], yet these thiols are unable to substitute for GSH in supporting DNA SSb repair [379]. In contrast to these results, Chinese hamster ovary cells whose non-protein thiols are depleted by diethylmaleate show no differences in the rate of repair of SSb induced by X-rays

[151]. Thus, the role of endogenous thiols in the repair of DNA damage must be studied further to clarify these conflicting findings.

Exogenous compounds have recently been investigated for their ability to induce the enzymes responsible for DNA repair. This property has been documented for a number of compounds which stress cells, including hydrogen peroxide [115], methylmethane sulphonate [62], nickel compounds [388], 1,6-dinitropyrene [91] and mercury [388]. The induction of DNA repair capacity has also been reported for the sulfur-containing radioprotector WR-2721 [384,385]. In contrast, cysteamine was found to inhibit DNA polymerase I-directed repair synthesis [66]. Cysteamine decreased the extent of rejoining of DNA SSb in GSH-proficient cells [316] but was able to substitute for glutathione in GSH-dependent SSb repair in GSH-deficient fibroblasts [136]. Thus, it appears that thiols are involved in certain types of DNA repair (though possibly not all types), and further research may point to new compounds able to stimulate the repair of radiation-induced DNA damage.

IV. Therapy of Radiation Damage.

In this section, the question: "What post-irradiation actions can be taken to ameliorate radiation injury?" is briefly considered. In general, therapeutic strategies after radiation exposure depend upon the dose received. At exposures above about 2000 rads, there is no effective treatment at the present time for the CNS-syndrome produced. At lower doses which can produce the acute effects of the gastrointestinal radiation syndrome, provision of symptomatic relief and physiological support must take priority over other considerations. If the patient survives this syndrome or does not experience it, then support of the functions and recovery of any hematopoietic injury can be provided. Beyond treating symptoms of these acute radiation syndromes, it should eventually be possible to provide therapeutic measures for enhancement of normal processes for DNA repair, including therapy to prevent expression of delayed effects of DNA lesions such as neoplasms.

A. Treatment of Gastrointestinal Failure.

Reviews of acute radiation syndromes and their supportive treatment are given by Bond *et al.* [70], Prasad [361], Pizzerello [351] and by Pizzerello and Witcofski [352]. As mentioned in Section I, the direct cause of radiation death depends on the dose absorbed. With whole body radiation doses from about 500 to about 5000 rads (above which the CNS syndrome causes more rapid death), gastrointestinal failure is typical. This syndrome is characterized by nausea, vomiting, anorexia, diarrhea, fluid electrolyte imbalance, increased vascular permeability, vascular collapse, and overwhelming infection as the gut wall is breached before destroyed mucosal cells can be regenerated. Spontaneous survival after such massive gastrointestinal injury is improbable.

Therapy for the gastrointestinal radiation syndrome should include: 1. Measures to combat infection (antibiotics, gamma globulin, sterile isolation, etc.); 2. Correction of fluid and electrolyte imbalances; and 3. Administration of other agents to ameliorate acute symptoms and treat tissue damage. Experimental work has identified certain agents which may eventually prove to be therapeutically useful. For example, Palladino *et al.* [339] described protection of chickens and mice from γ-irradiation mortality by use of certain proteolytic enzyme inhibitors. They suggest that the protection

was due to prevention of radiation-induced, protease-mediated increases in vascular permeability.

If the patient survives the gastrointestinal crisis (death can occur within a few days), then the more slowly expressed effects of hemopoietic failure can be treated.

B. Physiological Support during Recovery of Hemopoietic Functions.

Mammalian hemopoietic and lymphatic cells are quite radiosensitive compared to many other cell types. Whole body irradiation above approximately 200 rads (in humans) can severely depress the proliferating stem cell populations of these systems, with fatal results. Death is typically due to loss of the body's ability to combat infection and to blood loss consequences of acute depletion of the leukocyte and platelet populations. For doses below about 500 rads, therapeutic measures can be effective. Established therapeutic methods are designed to replace the impaired functions or cell populations until repopulation by surviving or administered cells can be accomplished. These measures include direct administration of fresh platelets, granulocytes, leukocytes, whole blood, bone marrow, spleen cells, etc. Antibiotics and sterile-tent isolation may be used during the period of increased vulnerability to infection.

These measures to sustain the patient during the bone-marrow recovery period can be supplemented by the administration of other agents which stimulate recovery of the hemopoietic system. Use of these agents, however, has been chiefly experimental. Agents with which some success has been achieved in irradiated animals include the following:

Interferon: Survival of hemopoietic tissue is reported to be enhanced by interferon [282,335] or by substances which can induce interferon synthesis, including sulfhydryl compounds, their phosphorylated derivatives, bacterial endotoxins, vasoactive amines (histamine, serotonin, mexamine), double-stranded RNA, and polysaccharides. With the currently increasing availability of microbiologically produced interferon, adequate evaluation of the utility of this agent in post-radiation therapy should now be feasible.

Tissue extracts: Active thymic polypeptides stimulate lymphocytopoiesis, increase immunological competence, stimulate metabolic recovery processes in thymus and liver of whole-body X-irradiated rats, and provide radioprotection [73,355,450]. In contrast, Czaplicki [111] reported that while embryonal calf thymus extract provided protection to mice if administered

before irradiation, it caused a rapid decrease in leukocyte count and decreased survival time if given after irradiation.

Glutaurine (γ-glutamyl-taurine), identified by Feuer et al. [156] as the radioprotective agent in parathyroid extract, partially prevents the radiation-induced decrease in bone marrow mitotic index. When injected as much as 3 hours after exposure, glutaurine supported 30-day survivals of 86%, compared with 50% for controls.

Endotoxin and Endotoxin Polysaccharides: Vigneulle and Baum [467] observed increased 30-day and 40-day survival rates in γ-irradiated mice given endotoxin immediately after exposure. The treatment caused an increase in the pool of myeloid precursor cells, allowing enhanced granulocytopoiesis.

Bacterial endotoxins consist of lipid and polysaccharide moieties, with the major endotoxic effects being due to the lipid. Nowotny et al. [322] have demonstrated that either the lipopolysaccharides or their hydrolyzed, nontoxic, polysaccharide-rich components are active inducers of bone marrow colony-stimulating factors in mice. They protect against lethal irradiation, have in vitro immune adjuvant activity, and show antineoplastic activity. However, the radiation protection with post-irradiation injection was markedly less than with administration before irradiation [322]. Work by this group (reviewed in [54]) indicated that the protective action is indirect, involving the release of mediators (by the lung and perhaps other tissues as well) into the serum. Thus, each of the four biological effects can be passively transferred by injecting serum from treated into untreated animals. Their work suggests that different fractions of the polysaccharide hydrolysates are responsible for the different biological activities. Injection of bacterial lipopolysaccharides after irradiation but immediately before bone marrow transfusion markedly improved spleen colony formation and erythroid differentiation in mice [426].

Hormones: Akoyev [10] emphasized the importance of the integrative functions of the endocrine system which aid recovery from radiation injury. He presented experimental results of treatment of irradiated animals with preparations from the thyroid gland, gonads, and adrenal cortex. He concluded that his and other data in the literature support the hypothesis that when post-irradiation endocrine deficiency begins to appear, administration of thyroid or adrenal cortical hormones can diminish hemopoietic deficits. Improvement of hemopoiesis, phagocytic capacity, and reaction to infectious

factors after hormonal treatment was demonstrated. Aggravated endocrine deficiency was correlated with increased expression of radiation injury whereas stimulation of endocrine function lessened that expression. Methylandrostadienolone is reported to stimulate postirradiation structural and metabolic repair in rats [395].

Other Agents: Synthetic lysophospholipid analogs of the naturally occurring 2-lyso-phosphatidylcholine were reported [58] to improve significantly the survival of X-irradiated mice, with the improvement being detectable even when treatment was given 6 hours after irradiation. Effective doses have been found to be without toxicity in clinical pilot studies. Although the mechanism of action is unknown, it is speculated that the central role of phospholipids in cell membrane structure and function, the influence of the analogs on normal DNA metabolism, or the possible interaction of phosphatidylcholine with chemical radicals may be involved.

Glucan, a potent reticuloendothelial stimulant and immune modulator which is isolated from the cell wall of Saccharomyces cerevisiae, enhances hemopoietic recovery in sub-lethally γ-irradiated mice when injected 24 hours postirradiation, although pretreatment is markedly more effective [354]. The protection mechanism is believed to be the stimulation of granulocyte production. Takeda et al. [434,435] and Yonezawa [488] report that single injections of ginseng extract to mice, rats, or guinea pigs within 2.5 hours of irradiation provided protection from bone marrow death. For example, when the injection was within 5 minutes of irradiation, 30-day survival was 76%, compared with 15% for controls.

An improved survival rate is produced by injection of tocopherol immediately after irradiation; no improvement is observed if the injection was postponed until 5 hours after irradiation [392]. Reports showing experimental postirradiation therapeutic effects of a number of other agents have been reviewed [351]. These include imidaxole, lipids (intraperitoneal or oral), lycopine, RNA, DNA, and erythropoietin. In each case, a large increase in survival rate for irradiated rats or mice was reported.

In most cases the clinical therapeutic potential of these various experimentally promising agents is yet to be evaluated. The variety of the agents found which can support hemopoietic recovery and the evidence [54] which suggests that the activity of the bacterial polysaccharides is mediated by the release of an endogenous agent encourages further efforts in identifying

such an endogenous agent which could prove to be therapeutically useful.

C. Reinforcement of Normal DNA Repair Processes.

Even if the absorbed radiation dose is low enough to preclude severe hemopoietic and gastrointestinal injury of the types discussed, radiation damage to DNA can produce serious delayed effects. Fortunately, precise and active DNA repair systems exist in cells. The subject of DNA repair has been reviewed [191,272,352] and is discussed in Section III.C.5.

Much investigation (summarized in [341]) has documented repair deficiencies in a number of heritable human disorders which are associated with increased cancer and/or increased sensitivity to radiation. The importance of DNA-repair to the normal organism is also made clear by the review of several human diseases associated with impaired DNA-repair activity by Friedberg et al. [166]. There can now be no doubt that repair processes are crucial to the determination of whether DNA lesions will ultimately be expressed.

At least some of the DNA repair processes are capable of responding to the stimulus of DNA damage with increased activity. The survival of irradiated cells has been shown in a number of cases to depend on postirradiation conditions [109]. Adjustment of these conditions to those which are optimal for repair of potentially lethal cellular damage therefore represents a therapeutic opportunity. Despite long-standing research attention to radioprotection through prevention of DNA injury, some radioprotective agents act in part, at least, through the enhancement of repair processes rather than by the prevention of DNA lesions. Indeed, work with repair-deficient mutant bacteria led Bresler [78] to postulate that repair enhancement is the main mechanism of radioprotectant action.

An experimental system in which repair of radiation-damaged DNA can be specifically studied (in the absence of complications introduced by effects of the radiation on the repair system itself) uses cultured mammalian cells which have been infected by radiation-damaged viral DNA. As reviewed by Defais [113], use of this system as a screening assay should allow identification of new candidate therapeutic agents and evaluation of DNA-repair enhancement by known radioprotectants (see §V.4).

Much of the available information concerning DNA repair and its modulation has been obtained with isolated cell systems. One can hope for, but not assume, clinical therapeutic potential for an agent which enhances repair

when added directly to cell cultures. Even in the experimental systems, the repair-enhancing agent usually must be added very soon after irradiation to be effective - too soon to seem practicable for treatment of most human exposures. Nevertheless, the accumulating evidence that carcinogenic and mutagenic DNA lesions are subject to repair by normal cellular mechanisms [191] is grounds for optimism. The results cited below, demonstrating that DNA repair can be stimulated by chemical intervention, may yield practical results since there are typically very long latent periods which extend between a mutagenic or carcinogenic transformation and its expression. Further work may eventually reveal practical approaches to DNA repair therapy of radiation injury. Specific agents showing experimental post-irradiation effects on DNA repair include:

Thiols: Riklis et al. [385] demonstrated that WR-2721 or mercaptopropionylglycine causes an increase in post-irradiation DNA repair, as represented by labeled thymidine incorporation by cultured hamster cells. Observations led them to conclude that these agents act not only as antioxidants and radical scavengers but also act by affecting repair processes.

Certain cellular repair systems may be thiol dependent. Research by Révész and co-workers [135,137,383] shows that post-irradiation incubation of cultured GSH-deficient fibroblasts with dithiothreitol or 2-mercaptopropionylglycine supports increased DNA repair. Post-irradiation addition of 1,4-dithiothreitol to leukocyte cultures was reported by Bick and Brown [63] to decrease chromosomal damage in hamster and Potorous cells by 36% and 46% respectively. The most significant effect is in the first 30 minutes after irradiation. In view of the short half-lives of free radicals, the reports of post-irradiation protection (continuing in some cases for hours) by free radical scavengers is surprising and suggests that there may be a delayed, post-irradiation production of free radicals.

Exogenous enzymes: Superoxide dismutase, recognized as being radioprotective through its prevention of superoxide-induced lesions [323], was reported by Simonyan [414] to improve survival of X-irradiated rats when given intraperitoneally thirty minutes after irradiation. Surprisingly, postirradiation administration is more effective than was administeration before irradiation.

Singh and Singh [415] suggest that the administration of exogenous repair enzymes, already demonstrated as effective in isolated cell systems,

and use of agents which induce synthesis of endogenous repair enzymes may have potential as therapeutic approaches to radiation injury.

Exogenous enzymes have also been shown to reduce the spontaneous aberrations in cells from Fanconi's and Bloom's syndromes [147], two of the heritable human conditions linked with increased radiation sensitivity and increased DNA damage. These observations led to the suggestion that the defect in the inherited conditions may be a decreased capacity for radical scavenging rather than deficient repair capabilities [147,169]. Presumably, an analogous hypothesis can be invoked as an explanation of the post-irradiation protection by radical scavengers: if irradiation injures the cell's basal radical scavenging capacity, neither a second generation of radiation-induced radicals nor impaired repair mechanisms need be hypothesized.

Other agents: Olontseva [325] attributes the protective effect of purified spleen extract, given one hour after irradiation of mice and hamsters, to the presence in the extract of highly active DNase I inhibitor. Borek [72] reported that selenium and retinoids, added to cell systems after radiogenic or chemically induced neoplastic transformation, suppresses the expression of that transformation.

D. General Principles.

Akoyev [10] reviewed some of the general hypotheses concerning radiation recovery processes (as of 1970) and reported additional experiments undertaken to explore some of the questions raised by these hypotheses. From his review of available information and his own work, Akoyev developed several general principles concerning mammalian recuperation from radiation injury. These include the facts that recovery occurs during, between and following radiation exposures and is proportional to the absorbed dose of radiation that does not inhibit the repair processes themselves. Radiation damage repair is proportional to the metabolic rate of the species and occurs on all levels of biological organization (molecular, cellular, tissue and organ levels). Finally, the rate of repair in various tissues parallels the rate of damage expression (i.e. bone marrow is faster than gastrointestinal tissue which is faster than nerve, muscle, etc.).

E. Summary.

Current treatment strategies for radiation injury can be said to be on a physiological basis. They are relatively effective for treatment of the bone marrow suppression and other symptoms of hemopoietic failure. However,

strategies appear merely symptomatic and supportive for treatment of the gastrointestinal syndrome, and are without effect in treatment of the CNS syndrome. Strategies remain in the realm of basic experimentation for reinforcement of DNA repair processes. The multiplicity of agents which have shown experimental promise for augmentation of bone-marrow recovery and DNA repair suggests that clinical advances in the near term can be expected in these areas.

V. Recommendations for Future Work.

In this section we present recommendations for future studies in the field of chemical radioprotection. We have identified six general areas in which we feel that additional work will lead to profitable advances in our understanding of the mechanisms of action of radioprotective drugs. These six areas are presented, according to our opinion, on a scale of decreasing priority.

1. Pulse Radiolysis.

The pulse radiolysis technique is providing information about the nature of the free radicals formed by ionizing radiation that is essential for the understanding of free radical interactions with cellular constituents. The measurement of radical yields, radical reaction pathways and rates of reactions in defined chemical systems provides data that aid enormously in understanding the biological effects of free radicals. Improvement of radioprotectors will require a biochemical and genetic understanding of these effects in defined systems as well as in more complex biological systems.

2. Protein Thiols and Mixed Disulfides.

A growing body of evidence indicates that protein thiols participate in the regulation of cellular metabolic and genetic processes. Ionizing radiation damage via reduced oxygen species can alter the oxidation reduction state (redox state) of the pyridine nucleotides and the thiol disulfide status. The glutathione redox cycle utilizes NADPH reducing equivalents in the reduction of GSSG back to GSH with glutathione reductase after the reductive transformation of hydrogen peroxide to water by the oxidation of GSH to GSSG which is catalyzed by glutathione peroxidase. The limiting rate in the glutathione redox cycle appears to be the rate of formation of NADPH via the pentose phosphate pathway. Failure to reduce GSSG rapidly results in the formation of protein glutathione mixed disulfides. These and other protein mixed disulfides have an undetermined effect on the rate of metabolism and genetic events including progression through the cell cycle.

Research is needed on several aspects of protein thiols. Improved measurement is needed on the quantity of these protein thiols that can form protein mixed disulfides with low molecular weight thiols and disulfides by thiol disulfide interchange reactions. With such information it would be

logical to determine the rates of thiol disulfide transferase activity with the protein mixed disulfide for the conversion to protein thiols and glutathione mixed disulfides followed by thiol and GSSG formation and the consumption of NADPH reducing equivalents for reduction of GSSG to GSH. It is important to have this information to understand better the radioprotective effects of thiol containing agents. Cells possess a very dynamic energy-dependent process for the maintenance of homostasis of thiols and disulfides. The perturbation of this homeostasis by the administration of exogenous thiol- or disulfide-containing radioprotectors is likely to alter these protein thiol- or protein mixed disulfide-dependent processes. A key to developing better radioprotective agents may lie in the understanding of the physiological role of this process.

3. Metabolism and Distribution of Radioprotective Compounds.

Studies on the metabolism and distribution of radioprotective agents appear to be quite inadequate. In view of the long history of chemical radioprotection, it is surprising that certain aspects of the basic pharmacology of these compounds have not been investigated. The few studies of metabolism of sulfur-containing radioprotectors which have been attempted appear to be quite unreliable. Many early studies used radioactively labelled compounds which were used to investigate the tissue distribution of the compounds. However, only the presence of radioactivity was determined in most of these studies. Therefore, no information on the biochemical transformations and ultimate fate of the compound can be ascertained by such experiments. Without such information, meaningful structure-activity relationships are impossible to determine (See §VII.A). Furthermore, the development of radioprotectors in cancer therapy has been hampered by unexpected pharmacokinetics of promising drugs, sometimes leading to untoward responses in patients participating in clinical trials [69,174,240]. Therefore, and effort must be directed toward the investigation of the metabolism and tissue distribution of representative radioprotective drugs. Such investigations should focus on the biotransformation of representative drugs with the identification and quantification of metabolites both *in vitro* and *in vivo*. Investigations on the extent and kinetics of absorption, distribution, and excretion of the parent drug and its metabolites will assist in judicious clinical use of these drugs and in the development of more advanced radioprotectors.

4. **DNA Repair Induction.**

The human body continuously repairs DNA as expressed by excretion in the urine of 1-2 μmoles day^{-1} $person^{-1}$ of modified bases, principally thymine (Dr. Bruce Ames, personal communication, 1984). Since oxidative stress appears to modulate the level of these products in some instances, such indicators of DNA damage could be valuable indicators of level of DNA repair that may occur with individuals. If DNA repair induction occurs and tolerance to oxidative stress develops then urinary products could be biological markers for such changes.

The virus/cell system described by Defais [113] for studying DNA repair does not appear to have been utilized for screening of potential post-irradiation protectants. It would appear to be well adapted to this purpose. This system, involving irradiation and purification of the viral DNA before its use to infect non-irradiated cultured mammalian cells and observation of the viral-DNA repair by those cells, has a number of advantages for this application. Replicate samples of homogeneously damaged DNA preparations can be administered reproducibly to cells exposed to different agents and conditions. Repair success can be objectively measured by observation of repaired virus activity. Effects on repair of radiolesions, divorced from any effects on their prevention, can be observed. The method should allow convenient preliminary screening of potential therapeutic agents as well as evaluation of the repair component of the action of radioprotectants. Systematic testing by this method of previously identified radioprotectants should add significantly to our understanding of chemical radioprotection.

5. **The Effect of Radical Acceptors on the Yield of Glutathione Radiolysis Products.**

According to the scheme of Willson *et al.* [160,484] (see Figure 3, §II.C.1.c), glutathione thiyl radicals formed during molecular "repair" of target radicals by hydrogen atom donation react with progressively less noxious radical acceptors to detoxify the sulfur-centered radical. If this hypothesis holds, one should be able to demonstrate, under conditions in which direct oxidation of the ultimate electron donor (*e.g.*, cytochrome c or NADPH) does not take place by the ultimate oxidant (·OH or R·), that the radiochemical yield of GSO_2H, GSSG and other glutathione radiolysis products

are diminished by the presence of the ultimate electron donor. For example, if the radical reactions

(58) $$R^{\cdot} + GSH \longrightarrow GS^{\cdot} + RH$$

(59) $$GS^{\cdot} + \text{Cyt } c^{red} \longrightarrow GS^{-} + \text{Cyt } c^{ox}$$

are thought to result in cycling of GSH between reaction 58 (hydrogen atom donation) and reaction 59 (electron transfer), then the yield of other products of the radiolysis of glutathione

(60) $$GS^{\cdot} \xrightarrow{O_2} GSO_2H + GH + GSSG$$

should be reduced in the presence of reduced cytochrome c compared to the yield of these oxidation products in its absence. This experiment could be repeated at whatever levels of biological relevance the hydrogen donation reaction had been shown to occur (i.e. purified systems, DNA, whole cells, etc.).

6. Toxicity of Thiol Radioprotectors.

A large proportion of radioprotectors contain a thiol group or a potential thiol group. This class of compounds contains many of the most effective radioprotectors. As with many drugs, the utility of thiol radioprotectors is limited by their toxicity. The prototypic thiol radioprotector, cysteamine, cannot be used clinically because of this toxicity. However, few studies of the toxic effects of thiol compounds have been done which quantify these toxic effects. Little is known about the relationship, if any, between the toxic effects of thiol and their radioprotective activity. The mechanism of toxicity has recently been investigated for the thiol-containing amino acid cysteine [468,469]. These studies have recently implicated cysteine oxidation, with concommittant production of hydrogen peroxide, in the mechanism of cysteine toxicity [470]. Cysteine has been shown to be mutagenic in the Salmonella reversion assay [173], presumably by similar mechanisms. If the radioprotective efficacy of thiol compounds results from their consumption of oxygen during their oxidation, the toxicity of these compounds may be an extension of their therapeutic effect. On the other hand, should other mechanisms be implicated in the therapeutic limitations of these drugs, such as the ulcerogenic [85] and adrenocortical necrotic [293] actions of cysteamine or the hypocalcemic effects of WR-2721 [174], one may be able to

dissociate the radioprotective from the toxic effects of the drugs. A similar separation of the toxic from the radiosensitizing effects of congeners of misonidazole [3] has recently begun to yield some promising results [103]. An understanding of the factors contributing to the toxicity of thiol radioprotectors may yield similar benefits in the therapeutic indices of these drugs.

VI. Chemical Radioprotection: Research In Progress.

A. Major Topics and Current Principal Investigators.

Introduction. Because radioresistance and radiosensitivity are reciprocal variables, research directed toward one of these topics is also related to the other. Much current research, for example, is aimed at chemically increasing radiosensitivity of tumor cells. Since modification of radiosensitivity is equally a modification of radioresistance, research projects concerning radiosensitization were not uniformly excluded from this compilation: if results of such a research project might be expected to also provide information concerning radioresistance, it was included here. However, many radiosensitivity projects which were judged to have only marginal relevance to radioprotection (such as clinical trials of radiosensitizers or investigation of radiosensitizer toxicity and pharmacology) were omitted.

In this section, projects related to eight topics are referenced by the principal investigator's surname. Further information concerning specific projects can be found in Section B.

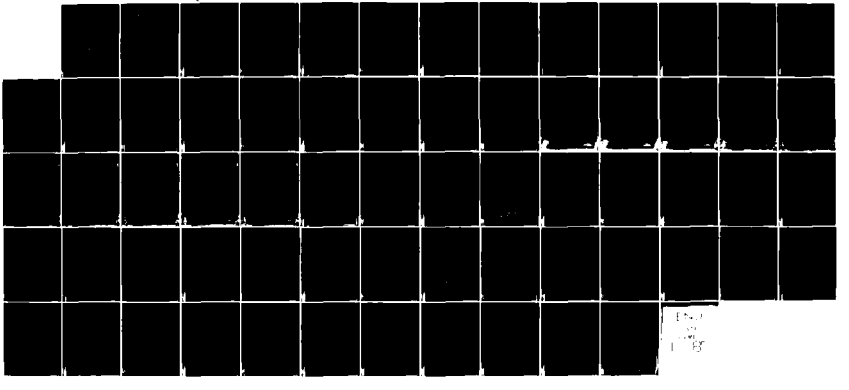
1. Radiation Damage: Basic Nature and Mechanisms.
Investigators: Adelstein, Bedford, Bernhard, Burns, Cole, Cress, Curtis, Denman, Dewey, Epp, Ewing, Fanburg, Geard, Gillette, Gregg, Griffiths, Griggs, Henner, Jose, Katz, Keng, Koval, Kubitschek, Lawrence, Leeper, Lett, Matney, Oleinick, Powers, Ramey, Remsen, Rich, Rodgers, Rosenberg, Roti Roti, Schneiderman, Setlow, Sinclair, Sowby, Smith, Stuart, B Sutherland, R Sutherland, Taylor, Tobias, Wallace, Wheeler, Withers, Zimbrick.

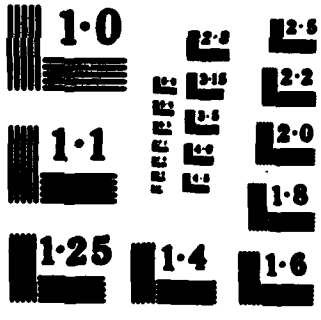
2A. Radiation Resistance/Sensitivity: Basic Nature and Mechanisms.
Investigators: Bedford, Biaglow, Ducoff, Gregg, Koval, Paterson.

2B. Radiation Resistance/Sensitivity: Temperature Effect.
Investigators: Bowden, Coleman, Cress, Denman, Dewey, Geard, Gerner, Hahn, Hall, Hofer, Kim, Leeper, Lett, Plenk, Schneiderman, Song.

2C. Radiation Resistance/Sensitivity: Oxygen Effect.
Investigators: Agrawal, Biaglow, Epp, Geard, Gupta, Hofer, Kim, Ling, Meistrich, Moulder, Mulcahy, Plenk, Remsen, Rich, Song, Suit, Tobias.

2D. Radiation Resistance/Sensitivity: Other Chemical Modulation.
Investigators: Agrawal, Arthur D Little, Inc., Bardos, Barkley, Biaglow, Brown, Chil Hosp Philadelphia, Ciborowski, Cole, Coleman, Epp, Ewing, Fanburg, Fu, Geard, Gillette, Gregg, Hagan, Hall, Henner, Hickman, Hofer, Infante, Jordan, Kligerman, Krohn, Leeper, Megaw, Meistrich, Mitchell, Moulder, Mulcahy, No Calif Cancer Prog, Parthasarathy, Phillips, Powers, Ramey, Research Triangle Inst., Rich, Richmond, Rosenthal, Sanders, Savarese, Schneiderman, Sodicoff, Song, Sridhar, Stuart, Suit, R Sutherland, Urtasun, Utley, Yuhas, Zimbrick.




1·0
1·1
1·25
1·4
1·6
1·8
2·0
2·2
2·5

3A. Radiation Repair/Recovery Processes: Basic Nature and Mechanisms.
Investigators: Brent, Burns, Cress, Denman, Ducoff, Evans, Fluke, Griffiths, Griggs, Hadden, Hanawalt, Henner, Howard-Flanders, Humphrey, Keng, Koval, Lawrence, Lett, Meyn, Oleinick, Paterson, Prakash, Ramey, Ramsen, Rich, Rupert, Setlow, Smith, B Sutherland, Taylor, Wallace, Wheeler.

3B. Radiation Repair/Recovery Processes: Chemical Modulation.
Investigators: Adler, Alving, Bardos, Brent, Gillette, Ling, Mulcahy, Remson, Setlow, Smith, R Sutherland.

4: Research on Methodology For Use In Radioprotection Studies.
Investigators: Brent, Ciborowski, Jordan, Lange, Matney, Megaw, Moss, Paterson, Wallace, Weinreb, Zimbrick.

B. Research Projects in Areas Relevant to Chemical Radioprotection.

Introduction: Listed on the following pages are some specific current or recent research projects relevant to chemical radioprotection funded by U.S. Government Agencies. It is emphasized that for some projects, the radioprotection component may represent only a secondary objective of the research. Entries given, when available, for each project are as follows:

PI/ORG: This entry identifies the principal investigator and organization with which the project is associated.

TITLE: This entry gives the title of the project.

SUMMARY: This entry gives brief characterization of that portion of the research project related to chemical radioprotection, to the extent that such information was available to us. In most cases, these entries consist of relevant selections and summaries from larger abstracts provided by the researchers or other sources.

SPONSOR: This entry indicates the source of funding for the research.

TOPICS: These codes refer to the eight major topics listed in section A.

SOURCES: The Sponsor names are followed by letters indicating the source of our information concerning the project. These sources are as follows:

A. CRISP (the U.S. Public Health Service's "Computer Retrieval of Information on Scientific Projects" file), a computer-based information system concerning all USPHS-supported grants and contracts and intramural research at the National Institutes of Health. We are grateful to Louis J. Parkhurst, Technical Information Specialist, for the search of CRISP on the topics "radioprotective agents" and "radiosensitizers", performed 2/23/84.

B. Federal Research in Progress (Unabridged) data base of Dialog Information Services, search performed 20 Feb, 1984.

C. RECON RIP data base of the Department of Energy. We are grateful to Axel Ringe, Science and Technology Division, DOE, for providing us with a search of this data base performed in February, 1984.

D. Commerce Business Daily (Dialog Data Base) - search performed Feb 22, 1984. The four contracts retrieved from this data base were listed in CBD as being in the process of negotiation. It is assumed that negotiations were successfully completed but no additional information is available for them. Therefore, they are listed separately here rather than in the main body of the following compilation.

CONTRACTS LISTED AS UNDER NEGOTIATION

PI/ORG: Arthur D Little, Inc., Cambridge, MA
TITLE: In vivo screening of radioprotectors.
SPONSOR: U.S. Army Med Res Dvlpt Command, Fort Detrick, MD/D TOPICS: 2D

PI/ORG: Children's Hosp, Philadelphia, PA
TITLE: Development of central nervous system radioprotectors
SPONSOR: U.S. Army Med Res Dvlpt Command, Fort Detrick, MD /D TOPICS: 2D

PI/ORG: Northern Calif Cancer Program, Palo Alto CA
TITLE: Screening of radiosensitizers and radioprotectors.
SPONSOR: Natl Cancer Inst, NIH /D TOPICS: 2D

PI/ORG: Research Triangle Inst., Research Triangle Park, NC
TITLE: Development of new prophylactic radioprotective agents.
SPONSOR: U.S. Army Med Res Dvlpt Command, Fort Detrick, MD /D TOPICS: 2D

PI/ORG: Adelstein, S James; Shields Warren Rad Lab, Harvard Univ, Boston MA
TITLE: Macromolecular radiation effects.
SUMMARY: Radiochemical alterations of amino acids, protein, and DNA in cultured hamster lung fibroblasts are studied.
SPONSOR: Natl Inst Arth, Diab, Digest and Kidney Dis /B TOPICS: 1

PI/ORG: Adler, HI; Oak Ridge National Laboratory, Oak Ridge TN
TITLE: Microbial mutagenesis and cell division.
SUMMARY: One objective is to explain the previously observed promotion by membrane preparations of recovery of irradiated bacteria.
SPONSOR: Dept Energy /C TOPICS: 3B

PI/ORG: Agrawal, Krishna C; Tulane Univ. Sch. Med., New Orleans, LA
TITLE: Development of antitumor and radiosensitizing agents.
SUMMARY: An objective is to develop and test a radiosensitizer for hypoxic tumor cells. Nitroimidazole analogs are synthesized and tested in vivo and in vitro.
SPONSOR: Natl Cancer Institute /A TOPICS: 2C,2D

PI/ORG: Bardos, Thomas J; SUNY at Buffalo, Amherst NY
TITLE: Chemical and biological studies in cancer chemotherapy.
SUMMARY: Objectives include determination of structure-activity relationships for radiosensitizing agents, design of new agents, and study of interaction of selected agents with nucleic acids and with DNA-repair processes.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D,3B

PI/ORG: Barkley, Howard T; Univ of Texas, Houston TX
TITLE: Radioprotective effects of prostaglandin inhibitors.
SUMMARY: This is a sub-project of a larger research program grant.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Bedford, Joel S; Radiology, Colorado State Univ, Fort Collins CO
TITLE: Dose and time factors in cellular radiosensitivity.
SUMMARY: The objectives are a better understanding of the nature of lethal lesions in irradiated cells and of the interrelationships of factors influencing radiosensitivity. Dose-rate and dose-fractionation effects are emphasized.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,2A

PI/ORG: Bernhard, William A; Dept Radiation, Univ Rochester, Rochester NY
TITLE: Effects of ionizing radiation on nucleic acids.
SUMMARY: The objective is to determine the chemical mechanisms by which ionizing radiation causes alterations in the primary structure of DNA, initially by developing a set of rules for prediction of DNA/free radical events.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1

PI/ORG: Biaglow, John E.; Case Western Reserve Univ., Cleveland OH
TITLE: Modification of X-ray response of anoxic-hypoxic cells.
SUMMARY: The nature of radiosensitization by mitogenic hormones of hypoxic tumor cells is studied. The effect of cellular thiols on radiosensitization by insulin and effects of drugs which influence endogenous non-protein thiols are investigated.Agents studied include epidermal growth factor, phorbol esters, rotenone, antimycin A, metronidazole, and misonidazole.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2A,2C,2D

PI/ORG: Bowden, George T; Univ Arizona, Rad'n Oncology Div, Tucson AZ
TITLE: Interaction of hyperthermia with radiation and drugs.
SUMMARY: (see summary under Eugene Gerner).
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2B

PI/ORG: Brent, Thomas P; St Jude Children's Research Hosp, Memphis TN
TITLE: Enzymes and reactions for repair of DNA in human cells.
SUMMARY: Objectives include identification and characterization of enzymes and reactions involved in excision-repair of DNA in human cells exposed to radiation or alkylating agents (using purified repair enzymes) and screening of cell extracts from individuals with "DNA repair syndromes" for repair enzyme deficiency.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 3A,3B,4

PI/ORG: Brown, John M.; Stanford Univ., Stanford CA
TITLE: Experimental radiotherapy - Basis and modification.
SUMMARY: Two of the topics of this project are (a) the influence of common chemotherapeutic agents on radiation resistance of normal spinal cord, lung, and kidney tissue; and (b) the testing and enhancement of tumor cell radiosensitization by electron-affinic agents. The agents studied include BCNU, misonidazole, actinomycin D, adriamycin, bleomycin, cyclophosphamide, and cis-platinum.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Burns, FJ; NY Univ Med Ctr, Tuxedo NY
TITLE: Oncogenic action of proton and electron radiation on rat skin.
SUMMARY: Single and double strand DNA breaks are studied using single and multiple radiation doses with special reference to repair of oncogenic damage

and to a track theory of radiation damage.
SPONSOR: Dept Energy /C TOPICS: 1,3A

PI/ORG: Ciborowski, Linda J; Massachusetts General Hospital, Boston MA
TITLE: Micronucleus screening of DDC and other radioprotectors.
SUMMARY: An in vitro assay based on observation of micronuclei formation is evaluated for quantifying radiation damage and for use as a screening assay for radioprotective effectiveness.
SPONSOR: Natl Cancer Institute /A TOPICS: 2D,4

PI/ORG: Cole, A; Dept of Physics, Univ Texas System Cancer Ctr, Houston TX
TITLE: Radiation and biophysical studies on cells and viruses.
SUMMARY: Continuing objectives are to define the initial lesions induced by radiation and subsequent events leading to cellular responses, using cultured, synchronized mammalian cells, low and high LET radiations, and various drugs.
SPONSOR: Dept Energy /C TOPICS: 1,2D

PI/ORG: Coleman, C Norman; Stanford Univ Sch Med, Stanford CA
TITLE: Stanford University participation in the NCOG.
SUMMARY: Stanford is a participant in the Northern California Oncology Group, whose major purpose is to design and conduct clinical trials, including trials involving radiosensitizers, radioprotectors, and hyperthermia.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2B,2D

PI/ORG: Cress, Anne E; Radiation Oncology, Univ Arizona, Tucson AZ
TITLE: DNA protein crosslinks after hyperthermia and radiation.
SUMMARY: The molecular and cellular nature of the DNA protein crosslinks generated by hyperthermia and radiation are investigated, with testing of their relationship to lethality, replication, and repair.
TOPICS: Natl Cancer Inst, NIH /B TOPICS: 1,2B,3A

PI/ORG: Curtis, Stanley B.; Univ Calif., Lawrence Berkeley Lab., Berkeley CA
TITLE: Response of rat tumor cells to heavy ions.
SUMMARY: The research measures responses of cellular processes to high- and low-LET radiation.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1

PI/ORG: Denman, David L; Univ Cincinnati Hosp, Cincinnati OH
TITLE: Chromosomal damage induced by hyperthermia and radiation.
SUMMARY: Studies of molecular mechanism of killing of cultured hamster ovary cells by radiation and heat, including the roles of repair and cell cycle stage are carried out.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,2B,3A

PI/ORG: Dewey, William C.; Univ of Calif. San Francisco CA, Colorado State Univ, Fort Collins CO
TITLE: Molecular basis of radiosensitization by hyperthermia.
SUMMARY: The research is to determine which heat-induced lesions are lethal in themselves and which radiosensitize by interaction with X-ray-induced lesions. Effects of heat on the number of radiaton-induced single-strand DNA breaks are determined.
SPONSOR: Natl Cancer Inst, NIH; US Dept Agriculture /A,B TOPICS: 1,2B

PI/ORG: Ducoff, Howard S; Univ Illinois, Urbana IL
TITLE: X-rays increase insect longevity - Possible mechanism.
SUMMARY: The objective is to test a model explaining the increase in longevity of irradiated insects. The model hypothesizes that radiation damage to DNA induces higher levels of DNA-repair enzymes.
SPONSOR: Natl Inst of Aging, NIH /B TOPICS: 2A,3A

PI/ORG: Epp, Edward R.; Massachusetts General Hospital, Boston MA
TITLE: Radiation sensitization applied to cancer radiobiology.
SUMMARY: Effects and mechanisms of radiation damage and of chemical radiosensitizers, including oxygen and its interaction with other sensitizers, are studied in mammalian cells. Certain "shoulder- modifying" compounds and compounds believed to alter indirect damage to RNA are also studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2C,2D

PI/ORG: Evans, HH; Dept Radiology, Case Western Reserve Univ, Cleveland OH
TITLE: Carcinogenesis in mammalian cells.
SUMMARY: The objective is to determine the role of DNA repair in mutagenesis and carcinogenesis. Reactions to radiation of repair-proficient and repair-deficient cells are compared.
SPONSOR: Dept Energy /C TOPICS: 3A

PI/ORG: Ewing, David; Hahnemann Univ School of Med, Philadelphia PA
TITLE: Lethal damage from O2 and OH in irradiated cells.
SUMMARY: Earlier conclusions regarding hydroxyl radicals and their relationship to oxygen in lethal radiation damage are reevaluated. The conclusion that oxygen sensitizes by reacting at cellular sites formed by 'OH attack is tested.
SPONSOR: Natl Cancer Inst, NIH /A,B TOPICS: 1,2D

PI/ORG: Fanburg, Barry L; New England Med Ctr Hosp, Boston MA
TITLE: Radiation injury to pulmonary endothelium.
SUMMARY: Radiation damage to cultured lung endothelial cells is characterized and biochemical mechanisms of damage and protection are studied, using radioprotectants and radiosensitizers.
SPONSOR: Natl Heart, Lung, and Blood Inst, NIH /B TOPICS: 1,2D

PI/ORG: Fu, Karen K; Univ of Calif., San Francisco CA
TITLE: Chemical modification of low dose rate irradiation.
SUMMARY: An objective of the research is to obtain quantitative information on modification of low-dose irradiation effects by radiosensitizers and radioprotectors in normal and tumor tissue in vitro.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Geard, Charles R; Columbia Univ, New York NY
TITLE: Radiation cytogenetics.
SUMMARY: The overall research objective is the study of effects of low-dose ionizing radiation on mammalian cells and the biophysical interpretation of these effects. Studies of modification by environmental factors, including oxygen and temperature, of dose response relationships are included.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2D,2C,2B

PI/ORG: Gerner, Eugene W.; Univ of Arizona, Rad Oncology Div., Tuscon AZ
TITLE: Biochemical and cellular aspects of hyperthermia damage.
SUMMARY: The primary objective of the program of which this is a sub-contract is to determine efficacy in cancer therapy of hyperthermia, used alone and with radiation or chemotherapy. Molecular, physical, engineering, and clinical aspects of the problem are studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2B

PI/ORG: Gillette, Edward L; Colorado State Univ, Fort Collins CO
TITLE: Radiation repair of normal mammalian tissues.
SUMMARY: Quantitative responses of microvascular and other normal tissues to radiation, with or without other agents, are evaluated with specific attention given to the relative biological effectiveness of high-LET radiations and to loss of repair capability after high- or low-LET irradiation combined with other agents, including the radioprotector WR-2721.
SPONSOR: Natl Cancer Inst, NIH /A,B TOPICS: 1,2D,3B

PI/ORG: Gregg, Earle C; Case Western Reserve Univ, Cleveland OH
TITLE: Mutants and altered radioresponse of cells and tumors.
SUMMARY: Objectives include (1) determination of effects of very small doses and dose rates of both drugs and ionizing radiation and (2) identification of biochemical, biophysical, and cytological differences between established radioresistant mutant cell lines and their parental source.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,2A,2D

PI/ORG: Griffiths, T Daniel; Northern Illinois Univ, DeKalb IL
TITLE: DNA replication after insult with UV.
SUMMARY: The time course of UV effects on DNA synthesis and of recovery from these effects, and molecular events responsible for the effects and recovery from them are studied.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A

PI/ORG: Griggs, Henry G; John Brown Univ Biology Dept, Siloam Springs, AR
TITLE: Ultraviolet and ionizing radiation damage.
SUMMARY: Radiation-induced intracellular processes, chromosomal lethal lesions, and cell repair processes in synchronous cell cultures are examined.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A

PI/ORG: Gupta, Vicram; Univ Texas Med Branch, Galveston TX
TITLE: Effect of physiological oxygen levels on tumor therapy.
SUMMARY: The effect in cultured tumor cells of physiological vs. 20% oxygen concentrations are studied in the hope that the results will aid interpretation of cytotoxicity data from drug and radiation exposures.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 2C

PI/ORG: Hadden, CT; Univ Tennessee, Oak Ridge TN
TITLE: Repair and cell cycle response in cells exposed to environmental biohazards.
SUMMARY: Removal in bacteria of light-induced pyrimidine dimers as controlled by UVR genes is studied.UVR induced proteins and their properties are studied to clarify repair processes.
SPONSOR: Dept Energy /C TOPICS: 3A

PI/ORG: Hahn, George M; Stanford Univ School of Medicine, Stanford CA
TITLE: Modification of radiation response <u>in vitro</u>.

SUMMARY: Objectives include the development of information on how hyperthermia kills cells, on cell thermotolerance, and on the effects of thermotolerance on X-ray sensitivity in vitro and in vivo.
SPONSOR: Natl Cancer Inst, NIH /A,B TOPICS: 2B

PI/ORG: Hall, Eric J.; Columbia Univ, New York, NY
TITLE: Radiobiological studies related to high LET radiotherapy.
SUMMARY: Purposes of the research include: study of the mechanism of action of radiosensitizing electron affinic compounds, including several newly synthesized ones; study of interactions with naturally occurring polyamines of several chemotherapeutic agents; and interaction of these agents with hyperthermia and with the electron affinic sensitizers.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS:2B,2D

PI/ORG: Hanawalt, PC; Herrin Biol Labs, Stanford Univ, Stanford CA
TITLE: DNA repair mechanisms in living cells exposed to ultraviolet light or environmental by-products of energy conversion.
SUMMARY: Bacteria are used for analysis of post-irradiation recovery processes.
SPONSOR: Dept Energy, NIH /A,C TOPICS: 3A

PI/ORG: Henner, William D; Dana-Farber Cancer Inst., Boston MA
TITLE: Mechanisms of radiation carcinogenesis.
SUMMARY: The chemistry and enzymology of X-ray-induced DNA damage and of DNA repair in human cells are studied. Location and extent of strand breaks and the effects of oxygen and free radical scavengers are determined, as well as rates and extent of post-radiation repair.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2D,3A

PI/ORG: Hofer, Kurt G; Florida State Univ., Tallahassee FL
TITLE: Tumor cell hypoxia as a factor in cancer therapy.
SUMMARY: Studies are made of the significance of the oxygen effect to radiotherapy; means of modification of the radioresistance of hypoxic cells including simultaneous application of radiation and hyperthermia or radiosensitizing agents; enhancement of by tumor cell pH reduction of hyperthermic radiosensitization; and the mechanism (damage enhancement vs repair inhibition) of synergistic radiosensitization.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2B,2C,2D

PI/ORG: Howard-Flanders, Paul; Yale Univ, New Haven CT
TITLE: Crosslinking of nucleoproteins by radiation.
SUMMARY: The enzymatic mechanisms of post-replication repair and genetic recombination in E. coli are investigated.
SPONSOR: Natl Inst of General Med Sciences, NIH /B TOPICS: 3A

PI/ORG: Humphrey, Ronald M; Univ of Texas System Cancer Center, Houston TX
TITLE: DNA repair and recovery in the mammalian cell cycle.
SUMMARY: Objectives include determination of the biochemical basis of cell cycle dependent variations in response to radiation and chemical agents; definition of the relationship of certain DNA repair processes to mammalian cell survival and mutagenesis; and isolation of mutagen-sensitive variant cell lines that could be used to investigate the molecular basis of DNA repair.
TOPICS: Natl Cancer Inst, NIH /B TOPICS: 3A

PI/ORG: Gabriel A Infante; Catholic Univ of Puerto Rico, Ponce PR
TITLE: Radiation chemistry of biologically important compounds.
SUMMARY: One of the subprojects continues studies of radiosensitization.
SPONSOR: Div of Research Resources /A TOPICS: 2D

PI/ORG: Jose, Jule G; Sch Optometry, Univ Calif, Berkeley CA
TITLE: Aging and radiation effects on ocular metabolism.
SUMMARY: Alterations in UV-induced DNA, RNA, protein, mucopolysaccharide, and proteoglycan metabolism in lens, cornea, and conjunctiva of young and old rats are examined.
SPONSOR: Natl Eye Institute, NIH /B TOPICS: 1

PI/ORG: Jordan, Scott W; Univ of New Mexico, Albuquerque NM
TITLE: Computer-based analysis of renal radiation response.
SUMMARY: Studies include evaluation of computer-assisted morphometric analysis for demonstration of tissue radiation response and of effects of radiation sensitizing or protective agents.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D,4

PI/ORG: Katz, R; Dept Physics, Univ Nebraska, Lincoln NE
TITLE: Theory of RBE.
SUMMARY: A model of track structure and relative biological effectiveness is being used in an attempt to understand biological effects of low dose ionizing radiation.
SPONSOR: Dept of Energy /C TOPICS: 1

PI/ORG: Keng, Peter C; Univ Rochester, Rochester NY
TITLE: Phase specific DNA repair in irradiated tumor cells.
SUMMARY: The objective is to investigate DNA damage and repair of irradiated syncronized tumor cells at different phases of the cell cycle to determine whether relative rate or extent of repair is responsible for variations in cell survival during the different phases.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS:1,3A

PI/ORG: Kligerman, Morton M; Univ Pennsylvanis, Philadelphia PA
TITLE: Clinical radiation and drug protection with WR-2721.
SUMMARY: Dosage and effectiveness of the radioprotector, WR-2721, in patients undergoing radiotherapy are tested, alone and in combination with cis-platinum and cyclophosphamide. Some pharmacologic studies with radiolabelled drug are included.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Kim, Jae H; Sloan Kettering Institute, New York NY
TITLE: Cellular response to heat and radiation.
SUMMARY: Interactive effects of glucose concentration, ambient oxygen concentration, and temperature on survival of HeLa cells are studied.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 2B,2C

PI/ORG: Koval, Thomas M; George Washington Univ Sch Med, Washington DC
TITLE: Insect cells - A basis for radioresistance.
SUMMARY: The objective is to determine the basis of the extreme radioresistance of cultured TN-238 insect cells. Several aspects of radioresponse, repair, and recovery of the cells are studied.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS:1,2A,3A

PI/ORG: Krohn, Kenneth A; Univ of Washington, Seattle WA
TITLE: Radioprotective drugs - Mechanism and biological studies.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Kubitschek, HE; Argonne Natl Lab, Argonne IL
TITLE: Molecular, genetic, and cellular mechanisms of environmental and solar-UV mutagens.
SUMMARY: The nature of DNA lesions produced by environmental and energy-related mutagens is studied using bacteria. Effects of ultraviolet light are emphasized.
SPONSOR: Dept of Energy /C TOPICS: 1

PI/ORG: Lange, CS; Downstate Med Ctr, State Univ of NY, Brooklyn NY
TITLE: Biological effects of ionizing radiation at the molecular, cellular, and organismal levels.
SUMMARY: Studies of DNA breakage and repair depend on DNA size measurements. This project attempts to develop accurate methods for these measurements.
SPONSOR: Dept of Energy /C TOPICS: 4

PI/ORG: Lawrence, CW; Dept Rad Biol and Biophys, Univ Rochester, Rochester NY
TITLE: Molecular biology of radiation mutagenesis.
SUMMARY: Among the objectives is the study of the effects of new mutations in Saccharomyces cerevisiae on DNA repair.
SPONSOR: Dept of Energy /C TOPICS: 1,3A

PI/ORG: Leeper, Dennis B; Thomas Jefferson Univ, Philadelphia PA
TITLE: Interaction of hyperthermia, radiation and drugs in vitro.
SUMMARY: Cell response to hyperthermia and to drug/radiation combinations are studied, as well as studies of physics and dosimetry of high energy protons, electrons and neutrons.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2B,2D

PI/ORG: Lett, John T; Biochem & Rad, Colorado State Univ, Fort Collins CO
TITLE: Repair of radiation damage to cellular DNA.
SUMMARY: Studies in hamster ovary cells of repair of X-ray induced DNA strand breaks as a function of cycle position and temperature and of responses of individual chromosomes are be carried out.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,2B,3A

PI/ORG: Ling, C Clifton; George Washington Univ, Washington DC
TITLE: Oxygen radiosensitization at different dose rates.
SUMMARY: The oxygen effect as a function of dose rate and of oxygen concentration is systematically studied. Measurements of split-dose recovery kinetics of mammalian cells at low oxygen concentrations are included.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 2C,3B

PI/ORG: Matney, TS; Univ of Texas Health Science Ctr, Houston TX
TITLE: The effect of radiation-sensitive mutations and mutagens/carcinogens on bacterial recombination and mutagenesis.
SUMMARY: The project deals with development of bacterial systems which quanti tate different types of radiation- and chemical-induced genetic recombinatin and mutation.
SPONSOR: Dept of Energy /C TOPICS: 1,4

PI/ORG: Megaw, Judith M; Emory Univ, Atlanta GA
TITLE: Liposomal intraocular drug delivery in vitro and in vivo.
SUMMARY: Delivery of liposome-bound specific agents to intraocular tissues, including delivery of free-radical scavengers to protect the lens from development of radiation-induced cataracts, are investigated.
SPONSOR: Natl Eye Institute /A TOPICS: 2D,4

PI/ORG: Meistrich, Marvin L; Univ. Texas System Cancer Ctr, Houston TX
TITLE: Mutagenic action of cancer therapy on testis cells.
SUMMARY: This project concerning the sterilizing and mutagenic action of drugs and radiation on male germ cells includes studies of factors influencing cell sensitivity; investigation of methods of protection against sterilizing effects; and testing of radioprotectors.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2C,2D

PI/ORG: Meyn, Raymond E; Univ of Texas System Cancer Ctr, Houston TX
TITLE: Repair of radiation damage in vitro and in vivo.
SUMMARY: The objective is detailed understanding of mammalian cell DNA repair mechanisms for radiation damage.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 3A

PI/ORG: Mitchell, J B; Natl Cancer Inst, NIH, Bethesda MD
TITLE: Response of human hematopoietic precursor cells to halogenated pyrimidines.
SUMMARY: Data relevant to whether the radiosensitizing halopyrimidines act by being actually incorporated into cellular DNA to make the DNA less stable to radiation are experimentally obtained.
SPONSOR: Div of Cancer Treatment, NIH /A TOPICS: 2D

PI/ORG: Moss, Alfred J; Veterans Administration Med Ctr, Little Rock AR
TITLE: Post-irradiation repair of DNA by mammalian cells.
SUMMARY: A sensitive oxygen assay for direct determination of oxygen concentration in suspensions of irradiated cells is being developed for use in studies evaluating DNA damage and repair.
SPONSOR: Veterans Administration /B TOPICS: 4

PI/ORG: Moulder, John E.; Medical College of Wisconsin, Milwaukee WI
TITLE: Optimization of radiosensitizer use in radiotherapy.
SUMMARY: Effects of hypoxia, the radiosensitizer misonidazole, and radiation are studied and distinguished in neoplastic and normal rat tissues.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2C,2D

PI/ORG: Mulcahy, R Timothy; Univ of Rochester, Rochester NY
TITLE: Radiation sensitizers - Interactions with other modalities.
SUMMARY: The research tests the hypothesis that repair inhibition by carbamoylation is correlated with radiosensitizer enhancement of effects of nitrosourea chemotherapeutic agents. Chemical structure (carbamoylating and alkylating properties), cellular environment (e.g., oxygen concentration), and cell proliferative status are among the variables studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2C,2D,3B

PI/ORG: Oleinick, Nancy L; Case Western Reserve Univ, Cleveland OH
TITLE: Radiation-induced modification in protein synthesis.
SUMMARY: Radiation-induced DNA damage and repair are compared in active DNAs

of proliferating cells and less active DNAs of plateau-phase cells.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A

PI/ORG: Paterson, Malcolm C; Chalk River Nuclear Labs, Chalk River, Canada
TITLE: Radiosensitivity and DNA repair in cancer risk.
SUMMARY: Radiosensitivity and DNA repair in skin fibroblasts from patients known to be at high risk of cancer are evaluated.
SPONSOR: Div Cancer Cause and Prevention /B TOPICS: 2A,3A,4

PI/ORG: Parthasarathy, Rengachary; Roswell Park Mem Inst, Buffalo NY
TITLE: Stereochemistry of thiol-disulfide interchanges.
SUMMARY: The role of thiol-disulfide interchanges in radiation protection and carcinogenesis are studied, using X-ray diffraction techniques to study crystal structures of thiols and related compounds and complexes. Results will be useful in designing radioprotective agents.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Phillips, Theodore L; Univ Calif. Medical Center, San Francisco CA
TITLE: Evaluation of radiosensitizers and radioprotectors for cancer therapy.
SUMMARY: This is a sub-project of the general clinical research center grant.
SPONSOR: Div Research Resources, NIH /A TOPICS: 2D

PI/ORG: Plenk, Henry P.; LDS Hospital, Salt Lake City UT
TITLE: Radiation therapy oncology group.
SUMMARY: Activities which this cooperative clinical research project helps support include pioneering programs in use of increased oxygen tension and of hyperthermia with radiotherapy.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2B,2C

PI/ORG: Powers, Edward L; Lab Rad Biol, Univ Texas, Austin TX
TITLE: Physico-chemical studies of radiation effects in cells.
SUMMARY: The effects of added metal (Ag or Hg) on radiation sensitivity of bacteria and the behavior of a radical scavenger and oxygen are studied.
SPONSOR: Natl Inst General Med Sci, NIH; Dept Energy /B,C TOPICS: 1,2D

PI/ORG: Prakash, Satya; Dept Biology, Univ Rochester, Rochester NY
TITLE: Repair of DNA damaged by psoralen + 360 NM irradiation.
SUMMARY: Systems for repair of DNA damage induced by exposure of cell systems to psoralen plus UV light are characterized.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 3A

PI/ORG: Rasey, Janet S; Univ of Washington, Seattle WA
TITLE: Neutron radiobiology in support of radiotherapy.
SUMMARY: Biological dosimetry to determine RBE's for neutron beams are carried out in vitro and in vivo, with investigation of repair of sublethal and potentially lethal damage and protection by WR-2721 and related thiols .
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2D,3A

PI/ORG: Remsen, Joyce F; Univ Calif., Davis CA
TITLE: Radiosensitizers and DNA damage.
SUMMARY: The objective is to characterize effects of hypoxic-cell radiation sensitizers on formation and repair of radiation-induced DNA damage in mammalian cells. Thymine damage and DNA strand breaks are studied in aerobic

and hypoxic conditions.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,3A,3B,3C

PI/ORG: Rich, Tyvin A.; Harvard Medical School, Boston MA
TITLE: Hypoxic cell sensitizers - Radiosensitization, distribution, neurotoxicity.
SUMMARY: Damage interaction between radiation and cancer chemotherapeutic agents; development of a basis for use of hypoxic cell sensitizers during radiotherapy; genetic control of drug resistant cells and radiation response of those cells; long-term effects of radiation; and repair in mammalian cells of radiation damage are studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2C,2D,3A

PI/ORG: Richmond, Robert C; Dartmouth-Hitchcock Med Ctr, Hanover NH
TITLE: Radiation-chemical induction of mutagenesis.
SUMMARY: Effects of sensitizers on radiation-induced mutagenesis are studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Rodgers, Michael A; Ctr for Fast Kinetics Res, Univ Texas, Austin TX
TITLE: Electron transfer reactions in micro-heterogeneous media.
SUMMARY: Ways in which aggregates and interfacial regions in heterogenous media influence one-electron transfers between free radicals are characterized. The results will have significance for understanding and control of health effects of high energy radiations.
SPONSOR: Natl Inst of General Med Sciences, NIH/A TOPICS: 1

PI/ORG: Rosenberg, Robert C.; Howard Univ. School of Med, Washington DC
TITLE: Superoxide dismutase in normal and malignant cells.
SUMMARY: This is a sub-project of a biomedical interdisciplinary research project.
SPONSOR: Div of Research Resources, NIH /A TOPICS: 1

PI/ORG: Rosenthal, C Julian; SUNY Downstate Med Ctr, Brooklyn NY
TITLE: Radiosensitization and treatment of malignant tumors.
SUMMARY: This is a sub-project of the general clinical research center grant.
SPONSOR: NIH /A TOPICS: 2D

PI/ORG: Roti Roti, Joseph L; Univ Utah, Salt Lake City UT
TITLE: Radiation induced alteration of chromosomal proteins.
SUMMARY: The objective is to determine the role of chromatin in the radioresponse of mammalian cells.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1

PI/ORG: Rupert, Claud S; Univ of Texas, Richardson TX
TITLE: Repair of radiation-damaged nucleic acid.
SUMMARY: DNA repair mechanisms in irradiated cells are studied.
SPONSOR: Natl Inst General Med Sciences, NIH /B TOPICS: 3A

PI/ORG: Sanders, CL; Battelle Pacific Northwest Labs, Richland WA
TITLE: Inhaled transuranics in rodents.
SUMMARY: One objective is to examine anticarcinogenic factors that modify carcinogenic processes resulting from inhalation of transuranic elements. Anticarcinogenesis with vitamins is evaluated.
SPONSOR: Dept of Energy /C TOPICS: 2D

PI/ORG: Savarese, Todd; Roger Williams General Hosp, Providence RI
TITLE: Differentiation-induction in human colon cancer cells.
SUMMARY: An objective is to determine whether differentiation-inducing compounds can alter sensitivity of cultured or *in situ* tumor cells to X-rays.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Schneiderman, Martin H
TITLE: G2 cell killing and arrest of X-ray and drugs.
SUMMARY: (see under Dennis Leeper)
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2B,2D

PI/ORG: Setlow, RB; Brookhaven Natl Lab, Upton NY
TITLE: Radiation and chemical damage to DNA and its repair.
SUMMARY: Radiation damage to bacterial and vertebrate cells and kinetics of three types of repair are measured along with effects of chemical modulators of repair processes. The aim is to develop a molecular basis for biological dose-response curves at low doses.
SPONSOR: Dept of Energy /C TOPICS: 1,3A,3B

PI/ORG: Sinclair, Warren K; Nat'l Coun Rad Protec and Meas, Bethesda MD
TITLE: Radiation effects and exposure criteria.
SUMMARY: The research assesses available information in order to develop NCRP reports on (among other topics) biological aspects of radiation protection criteria and biological effects of magnetic fields.
SPONSOR: Food and Drug Admin, USPHS/A TOPICS: 1,2D

PI/ORG: Smith, Kendric C; Dept Radiology, Stanford Univ, Stanford CA
TITLE: Repair of radiation-induced lesions in DNA.
SUMMARY: Objectives of these studies in *E. coli* include characterization of the processes for UV radiation mutagenesis; determination of the source and nature of spontaneous mutations; and molecular description of post-replication repair processes.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A

PI/ORG: Smith, Kendric C; Dept Radiology, Stanford Univ, Stanford CA
TITLE: Molecular basis of radiation lethality.
SUMMARY: Radiation-sensitive *E. coli* mutants are used to study various aspects of radiation-induced cell death, including the mechanisms and control for repair of DNA damage, interactions and possible radiation-induction of different repair pathways, and drug inhibition of repair.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A,3B

PI/ORG: Sodicoff, Marvin; Temple Univ., Philadelphia PA
TITLE: X-ray therapeutic index for salivary glands.
SUMMARY: The objective is to widen the differences in sensitivities of normal and neoplastic salivary tissue by use of the radioprotector, WR-2721, and the radiosensitizer, Ro-07-0582. Effects of the compounds, alone and together, are studied. Other drugs, including isoproterenol and cyclic AMP, are evaluated as possible substitutes for the still-experimental WR-2721.
SPONSOR: Natl Institute of Dental Research /A TOPICS: 2D

PI/ORG: Song, Chang W.; University of Hospitals, Minneapolis MN
TITLE: Use of 5-thio-D-glucose in radiotherapy.
SUMMARY: Studies showing that 5-thio-D-glucose is specifically cytotoxic

toward hypoxic cells, that it sensitizes these cells to x-irradiation while protecting oxic cells from radiation damage, and that the hypoxic cell cytotoxicity of the compound is dramatically enhanced by moderate hyperthermia are extended.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2B,2C,2D

PI/ORG: Sowby, FD; Internat Comm on Radiological Protection; Sutton, UK
TITLE: Recommendations on radiation protection.
SUMMARY: Fundamental radiobiological and other data are critically examined in reviewing the Committee's 1977 recommendations and (in the late 1980's) drafting its next recommendations for national and regional radiation protection programs.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1

PI/ORG: Sridhar, Rajagopalan; Oklahoma Med. Res. Fndn., Oklahoma City OK
TITLE: Interactions of hypoxic cell radiation sensitizers.
SUMMARY: Interactions of electron affinic nitro radiosensitizers with certain mammalian enzymes are studied. Effects of catecholamines on their radiosensitization are measured.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Stuart, Marie J; Upstate Med Ctr, State Univ NY, Syracuse NY
TITLE: Effect of irradiation on vascular-platelet interactions.
SUMMARY: Effects of radiation on arachidonic acid metabolism in blood vessels and their modification by radical scavengers and antioxidants are characterized to elucidate biochemical mechanisms involved in radiation effects on vascular/platelet interaction.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,2D

PI/ORG: Suit, Herman D; Dept Radiation Med, Mass General Hosp, Boston MA
TITLE: Modification of tumor response to local irradiation.
SUMMARY: Enhanced chemical radiosensitization by combination of sensitizer treatment with hyperbaric oxygen is evaluated in nine murine tumors.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 2C,2D

PI/ORG: Sutherland, Betsy M; Brookhaven Natl Laboratory, Upton NY
TITLE: UV transformation, DNA repair in human cells and skin.
SUMMARY: Roles of wavelength, age, cell origin, growth schedule, DNA and DNA repair on a UV-induced transformation process in human cell cultures are evaluated.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A

PI/ORG: Sutherland, Robert M.; Univ Rochester, Rochester NY
TITLE: Combined radiotherapy-chemotherapy studies.
SUMMARY: Experiments investigate the basic properties and responses of tumor and normal tissues to radiation and drugs, including the kinetics of repair processes.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2D,3B

PI/ORG: Taylor, JH; Inst of Molec Biophys, Florida State Univ, Tallahassee FL
TITLE: Repair of lesions and initiation of DNA replication in vertebrate cells.
SUMMARY: An objective is to study replicons and determine why these sites are

particularly sensitive to ionizing radiation using DNA cloning techniques in frog eggs or mammalian cell cultures.
SPONSOR: Dept of Energy /C TOPICS: 1,3A

PI/ORG: Tobias, Cornelius A; Univ of Calif. Donner Lab., Berkeley CA
TITLE: Heavy ion radiobiology related to oncology.
SUMMARY: Responses of normal mammalian tissues and of tumor growth and kinetics to heavy-ion beams are studied. Modification of these responses by oxygen are also studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2C

PI/ORG: Urtasun, Raul C; Cross Cancer Institute, Edmonton, AB, Canada
TITLE: R.T.O.G. phase I, and III clinical studies.
SUMMARY: Among the areas to which this project in a cooperative clinical research agreement will contribute is that of chemical modification of radiation response, with studies at both the basic and clinical research levels.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Utley, Joella F; Univ Calif Medical Center, San Diego
TITLE: Biological analysis of sulfhydryl radioprotective drugs.
SUMMARY: A new, sensitive assay method for the radioprotective agent, WR-2721, is used to study: 1) the intracellular form and site of binding of the drug which may elucidate its mechanisms of action; 2) the rate of its reaction with radicals; 3) possible radiation-drug mutagenesis; 4) pharmacokinetics in normal and tumor tissues and their possible alteration by radiation.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D,4

PI/ORG: Wallace, Susan S; Dept Microbiol, New York Med Coll, Valhalla NY
TITLE: Modification of X-ray induced damages in phage T4.
SUMMARY: The objective is to define the molecular mechanisms involved in repair of X-ray induced DNA damage in bacteriophage.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 3A

PI/ORG: Wallace, Susan S; Dept Microbiol, New York Med College, Valhalla NY
TITLE: Repair of DNA damage induced by ionizing radiation.
SUMMARY: The objective is to elucidate molecular mechanisms involved in repair by E. coli and S. cerevisiae of radiation damaged phage DNA.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 3A

PI/ORG: Wallace, SS; Dept Microbiol, NY Med College, Valhalla NY
TITLE: Immunochemical approach to the study of DNA repair.
SUMMARY: The objective is to develop a simple immunochemical assay to quantify DNA lesions so as to facilitate the study of DNA repair.
SPONSOR: Dept of Energy /C TOPICS: 3A

PI/ORG: Weinreb, Steven M; Pennsylvania State Univ, University Park PA
TITLE: Synthesis of securinega alkaloids.
SUMMARY: Some alkaloids have shown antiradiation activity. Total synthesis of some of the Securinega is proposed.
SPONSOR: Natl Inst of General Med Sciences, NIH /A TOPICS: 4

PI/ORG: Weiss, Herbert; Sloan-Kettering Inst., New York NY
TITLE: Mechanism of radiation damage in cells.
SUMMARY: The early physicochemical events involved in radiation effects in cells are studied, using spores, bacteria, and mammalian cells plus radiation sensitizers and radioprotectors.
SPONSOR: Natl Cancer Inst, NIH /A,B TOPICS: 1,2D

PI/ORG: Wheeler, Kenneth T; Rhode Island Hosp, Providence RI
TITLE: DNA damage and repair in irradiated brain and brain tumor.
SUMMARY: The objective is to elucidate the molecular mechanisms differentiating DNA repair kinetics in normal cells and tumor cells after irradiation.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1,3A

PI/ORG: Withers, H Rodney; UCLA Ctr for Health Science, Los Angeles CA
TITLE: Radiobiology of normal tissues.
SUMMARY: Responses of normal tissues (bone marrow stem cells, hair follicle cells, and adrenal tubule cells) to multiple doses of radiation are quantified.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 1

PI/ORG: Yuhas, John M; Children's Hosp of Philadelphia, Philadelphia PA
TITLE: Radioprotectants and radiosensitizers in radiotherapy.
SUMMARY: The objective is to determine whether WR-2721, given before radiation and radiosensitizers, increases therapeutic effectiveness.
SPONSOR: Natl Cancer Inst, NIH /B TOPICS: 2D

PI/ORG: Zimbrick, John D.; Univ. of Kansas, Lawrence KS
TITLE: Spin-labeled platinum complexes for radiochemotherapy.
SUMMARY: A series of such complexes are synthesized and tested for modification of cell response to radiation. Molecular mechanisms of their actions are studied.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 2D

PI/ORG: Zimbrick, John D; Univ of Kansas, Lawrence KS
TITLE: Radiation biochemistry of DNA base damage.
SUMMARY: The overall objective is to obtain information on types of radiation-induced DNA base lesions in bacteria and the biological consequences of the lesions. Quantities and types of base lesions and effects on these of radiosensitizers and radioprotectors are determined.
SPONSOR: Natl Cancer Inst, NIH /A TOPICS: 1,2D

VII. Appendix B: Some Compounds Showing Radioprotective Activity.

This Appendix contains information on the types of compounds which have been tested for radioprotective activity. Included in Section A are some general comments on radiobiological structure-activity relationships (SAR) which have been deduced from tests of efficacy of a wide range of compounds. These tests have been performed by the Antiradiation Drug Development Program of the U.S. Army Medical Research and Development Command. The comments on SAR which are included here are an abbreviated version of a discussion of SAR included in reference [431]. Section B contains data extracted from literature reports which attempt to define the mechanisms of action of radioprotectors.

Several points should be kept in mind by persons using this Appendix. First, the bare conclusions of SAR derived from whole animal bioassay studies [431] provides no information on the absorption, distribution, metabolism and excretion of the test compounds, but is based only on the end effect radioprotective activity. Thus, some compounds judged in some assays to be without radioprotective effect may indeed exert radioprotective activity if adequately delivered to, or appropriately metabolized by, the radiosensitive tissue. Other compounds which are effective radioprotectors of cells may be metabolized or excreted before they can be delivered to the radiosensitive tissue and thus will appear to be without effect. Therefore, one must interpret bioassay-derived SAR with a note of caution when no data on the metabolism and pharmacokinetics are available. Secondly, for persons using Section B, it should be noted that many prominant radiobiologists have cautioned that such "objective" parameters as Dose Modifying Factors (DMF) obtained in tissue/cell culture systems and even in whole animal studies may vary greatly from one laboratory to another ([14] and references therein). The proper form of equations defining cell survival curves is a continuing controversy among radiobiologists. Even with data fit to the same equation, changes in the pre- or post-irradiation cell culture conditions markedly influences the observed cell survival [109,110]. Unsuspected variables which are uncontrolled [100] further complicate comparisons of data between laboratories. Thus comparison of data between different laboratories is generally ill-advised for quantitative purposes [14]. The table of compounds, rather than giving quantitative indications of absolute radioprotective activity, should be used to orient the reader to the types of compounds currently being tested and to

suggest references to begin further investigation about the work being done on that radioprotector.

A. Structure-Activity Relationships.

Since many non-thiol radioprotectors exert their effect through well-understood pharmacologic effects (e.g., serotonin, isoproterenol), the structure-activity relationships (SAR) of these compounds will not be discussed in this report. The finding of radioprotective activity for cysteine and cysteamine (2-aminoethanethiol) in the late 1940's and early 1950's stimulated an effort to identify structural analogs of these compounds which exhibit increased effectiveness and/or decreased toxicity. The general findings of these efforts [431] are summarized in this section.

The general rule which has emerged from intensive investigation of aminothiol radioprotectors holds that the necessary requirements for radioprotective activity are: (1) a two or three carbon backbone separating (2) a thiol or potential thiol and (3) a primary or secondary amino functional

$$\underset{(3)}{R - NH -} \underset{(1)}{C_2 - C_1 -} \underset{(2)}{SR'}$$

group. While this "rule" is surely an oversimplification, its precepts have generally been upheld with few exceptions. Modifications of each of these requirements will be considered in turn.

(1) The Carbon Chain: The optimal size of the carbon chain separating the amino and thiol functional groups is two carbons. Separating these functional groups by more than three carbons abolishes radioprotective activity. In the series of 3-aminopropanethiols, few compounds showed more than low activity and many were without effect. Certain hydroxylated compounds, especially those carrying a hydroxyl group at the 2 position, were active when the amino group carried an alkyl chain. 3-Aminopropylphosphorothioates followed this generalization, with 2-hydroxy-3-alkylaminopropylphosphorothioates showing some activity.

Among the amino(alkylethane)thiols, most substitutions resulted in the diminution or abolition of radioprotective activity. Activity was retained in the series 2-alkyl-2-aminoethanethiols (up to propyl substitution) and varied results were obtained in the 2-alkyl-2-methyl-2-aminoethanethiols. However, more extensive substitution, including involvement of carbocyclic

structures at the 2 position, abolished radioprotective activity. Alkyl substitution at the C_1 carbon was more hindered in terms of the size of permissable substitutions, with no activity beyond ethyl substitution at C_1. The involvement of both carbons of aminoethanethiol as part of a carbocycle (i.e., 2-aminocyclobutanethiol) retained activity, which diminished somewhat as the size of the carbocyclic ring was increased.

Functionalization of either R_1 or R_2 in the series $H_2NC(R_1R_2)CH_2SH$ dramatically altered the toxicity and/or radioprotective activity. Incorporation of thiol functions into these alkyl groups greatly increased the toxicity of the compounds. Hydroxylation of these alkyl groups increased the efficacy of the compound relative to the non-functionalized compound; the phosphorothioates of this series were generally highly active with relatively low toxicity.

(2) The Thiol Group: Early in the radioprotective drug development program, it became apparent that a thiol group or a potential thiol group was generally necessary for radioprotective activity. The nature of the "potential" thiol group influenced the relative activity and toxicity of the basic compound. Thus, blocking the thiol group in a manner such that it was metabolically unavailable (e.g., alkylation of the thiol to produce a thioether) eliminated radioprotective activity. However, blocked forms of thiols (e.g., thiosulfates (Bunte salts), phosphorothioates, disulfides[3], or other derivatives from which free thiols may be formed metabolically) were active to varying degrees, both in absolute dosage and relative to the toxicity of the compound. Altogether, about 50 different sulfur blocking groups were tested in varying degrees for their ability to latentiate the thiol and alter the relative potency of the parent aminothiol. In general, the three thiol derivatives mentioned above were found to be most promising. The blocked thiols have generally been considered to be prodrugs; the free thiol was thought to be the active form at the site of action. One might assume that the function of the blocking group was to alter the pharmacokinetics or the rates of metabolism and excretion of the drugs. While this is a plausible hypothesis, extensive data to support this general

[3] Note that hydrolysis of most pro-thiols yields one mole of thiol per mole of pro-thiol whereas on reduction, disulfides yield two moles of thiol per mole of disulfide.

conclusion is remarkably lacking; more detailed studies of the structure/activity relationships between the different series of blocked thiols must be compared with data on the pharmacokinetics of these drugs before this general conclusion can be accepted.

(3) The Amino Group: Various structures may be attached to the amino group of the basic aminoethanethiol structure with no deleterious effect on radioprotective activity. The N-(n-alkylamino)ethanethiosulfuric acid series ($RNHCH_2CH_2SSO_3H$) affords an interesting pattern of activity, with full activity where R includes a straight chain of up to three carbon atoms. The next four compounds (R = C_4 through C_7) were inactive, but modest activity returned when R = C_8 to C_{10}; further extensions were inactive or exhibited only very low activity. Branched chain hydrocarbons attached to the amino functional group were generally representative of the activity of the unbranched homologue. Tertiary amines (dialkylaminoethanethiols) were without activity, including those compounds in which the nitrogen atom was part of a heterocyclic ring.

Introduction of a phenyl group onto the alkylaminoethanethiosulfuric acids (resulting in compounds of the series $C_6H_5(CH_2)_nNHCH_2CH_2SSO_3H$) produced inactive compounds for n = 0 - 2, but compounds showing good activity for n = 4 and 5. Incorporation of alkyl substitutients on the phenyl ring of 4-phenyl-n-butylaminoethanethiosulfuric acid increased the toxicity of the compound dramatically, while substitution with a methoxy group produced higher radioprotective potency.

Hydroxylation of the alkyl chain in the N-alkylaminoethanethiols reduced the toxicity of the compound irrespective of the position of the hydroxyl group on the alkyl chain. In general, the introduction of this group had little effect on or reduced the radioprotective potency when compared to the nonhydroxylated homologue. Polyhydroxylation further reduced the toxicity, and in some cases allowed testing for potency at higher doses, revealing good activity.

Many other substitutients on the amino functional group have been tested for radioprotective activity, including phenoxyalkyl-, cycloalkylalkyl-, heterocycloalkyl-, pyridylalkyl-, quinolyloxyalkyl-aminoethanethiols and their blocked thiol derivatives. Varying activity was encountered, not always in orderly sequence or in relation to their expected pharmacokinetic behavior.

Perhaps the best known alkylamino functionalization is the group of aminoalkylaminoethanethiols and phosphorothioates [$RNH(CH_2)_nNH(CH_2)_mSR'$], represented by the prototypic compound WR-2721 [S-2-(3-aminopropylamino)-ethylphosphorothioic acid, R = H, R' = PO_3H_2, n = 3, m = 2]. In general, the phosphorothioates in this group were more active than the thiols and were less toxic. Alkylaminoalkylaminoethanethiols or phosphorothioates were inactive when the terminal alkyl chain contained more than one carbon atom. Varying the length of the alkyl chain between the amino groups produced a peak of activity at n = 3. In contrast to the thiols, phosphorothioates in this class could be hydroxylated in the aminoalkylamino group without loss of activity. As noted above, the compounds in which n = 2 and m = 3 were of comparable activity when R' = H or PO_3H_2.

In view of the above requirements for a (potential) thiol and a primary or secondary amine, it is interesting that N,N'-bridged amino(bis(ethanethiols)) and derivatives [$R'S(CH_2)_2NH(CH_n)NH(CH_2)_2SR'$] were almost uniformly ineffective. The only exceptions were the diphosphorothioates (R' = PO_3H_2) where n = 3 or 4.

Other Compounds: Certain other compounds not patterned after the basic aminoethanethiol structure have also been tested for radioprotective activity. Mercaptoacetamidines, generally tested as the thiosulfates, are somewhat more effective than the corresponding aminoethyl analogs, but are also more toxic. In contrast to the aminoalkylthiol series, amidines containing more than 2 carbons in the primary alkyl chain were active, albeit with somewhat lower activity than the shorter chain compounds.

Guanidinoalkylthiols and derivatives [$RNHC(=NH)N(R_1)(CH_2)_nSR'$ Y = H, SO_3H, or disulfide] showed little promise in terms of radioprotective activity. This result was somewhat unexpected, since the parent compounds [$H_2NC(=NH)NH(CH_2)_nSH$, n = 2 or 3] are generally considered the active components of the isothiuronium compounds AET (Aminoethylisothiuronium) and APT (Aminopropylisothiuronium) which have the structure $H_2N(CH_2)_nSC(=NH)NH$, n = 2 and 3, respectively. This result may demonstrate one of the hazards of inferring too much from the structure-activity relationships when pharmacokinetic behavior is not, or is only briefly, considered.

B. Compound List.

Compound	Dose	Biological Material	Radiation	Effect	Reference
Acetylthiourea	0.1M	*Shigella flexneri* Y6R	e^-;20 kR/min	P-air 1.7, P-N_2 1.1	[106]
Aminoethane thiol	20mM	V79 cells in culture	near UV radiation	2x increase in D_o value	[189]
Aminoethylisothiuronium BrHBr (AET)	2-5mM	mouse fibroblast L929 cells	fast neutrons 4-5 MeV;0.47-5.2 Gy	increased survival rate severalfold	[154]
Aminoethylisothiuronium BrHBr (AET)	0.45mM/kg	rat liver mitochondria	γ(7Gy)30 min after treatment with AET	"antagonized radiation induced increase in protein synthesis	[167]
Aminoethylisothiuronium BrHBr (AET)	300mg/kg i.p. 15min before	mouse bone marrow cells *in vivo* irradiation	^{60}Coγ 50-250 R	reduced frequency of polychromatic erythrocytes	[168]
Aminoethylisothiuronium BrHBr (AET)	2,4 or 5mM	mouse cells	^{60}Co-γ 0.24 Gy/s	DRF 2mM = 1.20 4mM = 1.27 5mM = 1.34	[155]
			4-5MeV neutrons	DRF 5mM = 1.00	
Chloral hydrate	0.3mg/g i.p.	MT tumors (mice)	X-ray to TCD_{50}	DMF 1.05	[407]
Cystamine	50moles/mole enzyme	aldolase	X-rays	enzyme inactivation (< 1) < DMF < 3	[177]
	50moles/mole enzyme	yeast alcohol dehydrogenase	X-rays	enzyme inactivation (< 1) < DMF < 3	
	50moles/mole enzyme	pancreatic α-amylase	X-rays	enzyme inactivation (< 1) < DMF < 3	
	50moles/mole enzyme	*B. subtilis* α-amylase	X-rays	enzyme inactivation (< 1) < DMF < 3	

Compound	Dose	Biological Material	Radiation	Effect	Reference
Cystamine	10^{-2}M	E. coli RNA polymerase) with σ-factor)	X-ray;1.75 kR/min (220kV,20mA,0.5mm Cu filter)	DRF 2 (calf thymus template)	[430]
	10^{-2}M	E. coli RNA polymerase) with σ-factor)	X-ray;1.75 kR/min (220kV,20mA,0.5mm Cu filter)	DRF 30 (T4 template)	
Cystamine	60mg/kg i.m. 20min before irradiation	adult male and female Wister SPF rats (180-230g)	^{60}Co-γ; 0.78 Gy/min	DRF 1.46	[257]
	50mg/kg i.p. 15min before irradiation	adult male and female Wister SPF rats (180-230g)	^{60}Co-γ; 0.386 Gy/min	DRF 1.79	
Cystamine	50mg/kg i.m. 15min before irradiation	adult male and female Wister SPF rats (180-230g)	^{60}Co-γ; 0.386 Gy/min	DRF 1.65	[257]
	50mg/kg i.p. 15min before irradiation	adult male and female Wister SPF rats (180-230g)	^{60}Co-γ; 0.369 Gy/min	DRF 1.44	
Cystamine	50mg/kg i.m. 15min before irradiation	adult male and female Wister SPF rats (180-230g)	^{60}Co-γ; 0.369 Gy/min	DRF 1.24	[248]
Cysteamine	150mg/kg	endothelial cells of rat cerebral cortex	^{60}Co-γ; 3 Gy/min	1.3	[446]
Cysteamine	75mM	Chinese hamster V79 cells (synchronized)	50 kV X-rays, LET = $(^{3}H)_{\infty}$90 keV/μm	DMF (G_1/S) 4.8 DMF (late S) 3.2	[67]
	75mM	Chinese hamster V79 cells (synchronized)	50 kV X-rays, LET = $(^{3}H)_{\infty}$170 keV/μm	DMF (G_1/S) 1.6 DMF (late S) 1.5	

Compound	Dose	Biological Material	Radiation	Effect	Reference
Cysteamine	50moles/mole enzyme	aldolase	X-rays	enzyme inactivation 2 < DMF < 6	[177]
	50moles/mole enzyme	yeast alcohol dehydrogenase	X-rays	enzyme inactivation 2 < DMF < 6	
	50moles/mole enzyme	pancreatic α-amylase	X-rays	enzyme inactivation 2 < DMF < 6	
	50moles/mole enzyme	B. subtilis α-amylase	X-rays	enzyme inactivation 2 < DMF < 6	
Cysteamine	1.6×10^{-3}M	calf thymus DNA	^{137}Cs;600 R/min	"weak protection"	[247]
	1.6×10^{-3}M	salmon sperm DNA	^{137}Cs;600 R/min	"weak protection"	
Cysteamine chloride	10^{-3}M	microsomes from liver cells of male Sprague-Dawley rats (180-200g)	^{60}Co γ-ray;80 kR	decreases V_{max} of ethylmorphine demethylation; increases V_{max} of cyt P-450-ethylmorphine interaction	[491]
Cysteine	50moles/mole enzyme	aldolase	X-rays	enzyme inactivation 2 < DMF < 6	[177]
Cysteine	50moles/mole enzyme	yeast alcohol dehydrogenase	X-rays	enzyme inactivation 2 < DMF < 6	[177]
	50moles/mole enzyme	pancreatic α-amylase	X-rays	enzyme inactivation 2 < DMF < 6	
	50moles/mole enzyme	B. subtilis α-amylase	X-rays	enzyme inactivation 2 < DMF < 6	
Cysteine	575mg/kg i.p.	rats	800R X-rays at 210 R/min	82% survival at 30 days vs. 10% survival in Controls	[343]

Compound	Dose	Biological Material	Radiation	Effect	Reference
Cysteine	0.1M	anoxic *Pseudomonas* spp.	γ	DRF 1.8	[80]
L-Cysteine	40mg/day	male mice	^{60}Co-γ	DRF 1.19 ± 0.05	[475]
Cystine	50moles/mole enzyme	aldolase	X-rays	enzyme inactivation 1.3 DMF < 4	[177]
	50moles/mole enzyme	yeast alcohol dehydrogenase	X-rays	enzyme inactivation 1.3 DMF < 4	
	50moles/mole enzyme	pancreatic α-amylase	X-rays	enzyme inactivation 1.3 DMF < 4	
	50moles/mole enzyme	*B. subtilis* α-amylase	X-rays	enzyme inactivation 1.3 DMF < 4	
2-Di(n-butyl)-germathiazolidine	4mM	human kidney T cells (culture)	X-rays	DRF 2.0	[471]
Diethylaminoreserpine (DL-152)	320mg/kg	mice	120 R/min (250 kV, 20mA hvl, 1.6mm Cu)	DMF intestine 1.15 skin 1.5 - 1.9 bone marrow 1.0 KHT tumor 1.7 EMT6 tumor 1.0	[267]
2-Diethyl-2-germathiazolidine	4mM	human kidney T cells (culture)	X-rays	DRF 2.7	[471]
Dimethylsulfoxide	1M	anoxic *Pseudomonas* spp.	γ	DRF 1.6	[80]
2,2-Dimethyl-thiazolidine	250mg/kg	mouse bone marrow colony forming units	γ-radiation	reduced radiation-induced inhibition of CFU growth activity	[271]
Glycerol	2M	anoxic *Pseudomonas* spp.	γ	DRF 2.0	[80]

Compound	Dose	Biological Material	Radiation	Effect	Reference
Guanylthiourea	0.3M 0.1M	Shigella flexneri Y6R Shigella flexneri Y6R	e^-;20 kR/min e^-;20 kR/min	P-air 2.3, P-N_2 1.14 P-air 1.9, P-N_2 1.3	[106]
bis-(2-Guanidoethyl) disulphide	1.6×10^{-3}M	calf thymus DNA	^{137}Cs;600 R/min	"strong protection"	[247]
bis-(2-Guanido-ethyl) disulphide	1.6×10^{-3}M	salmon sperm DNA	^{137}Cs;600 R/min	"strong protection"	[247]
O-(β-Hydroxyethyl)-rutosides	450mg/kg 30min before radiation	neonatal rat brain microvasculature	1 Gy/min (Piotron)	DMF 1.3	[262]
Imidazolidine thione	0.1M	Shigella flexneri Y6R	e^-;20 kR/min	P-air 2.1, P-N_2 1.7	[106]
Mercaptopropionyl glycine	20mg/kg	mouse liver nuclei	tritiated water 5μCi/gm B.W. 15-30 min after drug	44% reduction of abnormal mouse liver nuclei 3 days after radiation	[187]
2-(o-Methylphenyl)-thiazolidine	6mM	human kidney T cells (culture)	X-rays	DRF 1.6	[471]
1-Methyl-2-phenyl-thiazolidine	6mM	human kidney T cells (culture)	X-rays	DRF 1.8	[471]
D-Penicillamine	3gm/kg 60min before radiation	3-4 day old mice	^{60}Co-γ; 6-10 Gy	increased $LD_{50}/_{30}$ from 6.77 Gy to 8.28 Gy	[259]
D-Penicillamine	10mg/day p.o.	male rats	^{60}Co-γ; 25 Gy to right hemithorax	reduced lung pathology due to radiation	[353]
D-Penicillamine	10mg/day p.o.	male rats	^{60}Co-γ; 25 Gy to right hemithorax	reduced radiation-induced arterial perfusion defects in lungs	[473]
D-Penicillamine	10mg/day p.o.	male rats	^{60}Co-γ; 25 Gy to right hemithorax	prevented or reduced changes in lung enzymes caused by radiation	[474]

Compound	Dose	Biological Material	Radiation	Effect	Reference
D-Penicillamine	(1) 10mg/day (2)100mg/day	male mice	^{60}C-γ; 500-1000 R	(1) DRF 1.04 ± 0.04 (2) DRF 1.13 ± 0.04	[475]
D-Penicillamine	2mg/day	male mice	^{60}Co-γ; 10-20 Gy	comparison of LD_{50}/ 181-360 days gave DRF 1.2	[476]
Pentobarbital	0.06mg/g i.p.	MT tumors (mice)	X-ray to TCD_{50}	DMF 1.07	[67]
2-Phenylthiazolidine	8mM	human kidney T cells (culture)	X-rays	DRF 1.8	[471]
Structure 1 Structure 2 Structure 3	150mg/kg, LD_{50}=300 600mg/kg, LD_{50}=1200 600mg/kg, LD_{50}=1200	male mice male mice male mice	^{60}Co-γ; 40 R/min ^{60}Co-γ; 40 R/min ^{60}Co-γ; 40 R/min	DRF 1.4 DRF 1.3 DRF 1.4	[300]
Thiazolidine	8mM	human kidney T cells (culture)	X-rays	DRF 1.6	[471]
Thiocarbohydrazide	0.05M	*Shigella flexneri* Y6R	e^-;20 kR/min	P-air 1.8, P-N_2 1.1	[106]
5-thio-D-glucose	1.5g/kg	A/J female mice	2000-5000 rad	DMF 1.1 - 1.3	[402]
	1.5g/kg	A/J female mice	(220kV_p;15ma)	DMF 1.3 foot (0-40 days)	
	1.5g/kg	A/J female mice	117-124 rad/min	DMF 1.2 foot (60-90 days)	
Thiosemicarbazide	0.1M	*Shigella flexneri* Y6R	e^-;20 kR/min	P-air 2.4, P-N_2 1.7	[106]
Thiourea	0.3M 0.1M	*Shigella flexneri* Y6R *Shigella flexneri* Y6R	e^-;20 kR/min e^-;20 kR/min	P-air 3.5, P-N_2 2.2 P-air 2.3, P-N_2 1.5	[106]
Thiourea	0.2M	anoxic *Pseudomonas* spp.		DRF 2.1	[80]

Compound	Dose	Biological Material	Radiation	Effect	Reference
WR-2721	400mg/kg i.p.	C57 BL/6J male mice (20-25g)	^{137}Cs-γ; 117 R/min	DRF 1.75 (20min)	[248]
	400mg/kg i.p.	C57 BL/6J male mice (20-25g)	^{137}Cs-γ; 117 R/min	DRF 1.32 (60min)	
WR-2721	220mg/kg 15min before radiation	weanling mouse	250kVp X-rays	DRF 1.16 - 1.20 for renal growth protection	[412]
WR-2721	3mg/ml 30min	V79 spheroids in culture	^{137}Cs; 6.2 Gy/min	DMF 2.15 at 1% O_2	[132]
WR-2721	400mg/kg 30min before radiation	male mice	^{137}Cs-γ; 33-62 Gy at 917 R/min	DMF 1.5 for 5mm reduction in leg contractions	[214]
WR-2721	300mg/kg i.m. 15min before radiation	mice	^{60}Co-γ; 0.33 Gy/min	hemopoietic death DRF 2.14	[256]
WR-2721	400mg/kg 15min before radiation	female mice	^{60}Co-γ; 40 R/min	DRF 1.6	[238]
WR-2721	400mg/kg 30min before radiation	mice	^{137}Cs-γ; 9.17 Gy/min	DRF for tumor cells: 1.11 - 1.24	[306]
WR-2721	400mg/kg 15min before radiation	mice	^{137}Cs-γ; 9.17 Gy/min	DMF (jejunum) 1.64 (testis) 1.54 (fibrosarcoma) 1.28	[307]

Compound	Dose	Biological Material	Radiation	Effect	Reference
WR-2721	400mg/kg 30min before radiation	mice	^{137}Cs-γ; 9.17 Gy/min	DMF (hair loss) = 1.24	[308]
WR-2721	4mM	human fibroblast cell culture	^{137}Cs-γ	DMF for DNA strand breaks = 1.00	[386]
WR-2721	500mg/kg	EMT6 tumors in BALB/C mice	6 MeV X-rays 5 Gy/min	DMF 1.15 ± 0.04	[297]
WR-2721	500mg/kg	BALB/C mice	^{60}Co-γ; 200 R/min	hematopoietic death DMF 2.15	[104]
	300mg/kg	BALB/C mice	^{60}Co-γ; 200 R/min	gastrointestinal death DMF 1.65	
WR-2721	400mg/kg	rat parotid gland	X-radiation	DMF 1.92	[421]
WR-2721	400mg/kg	rat parotid gland	300kVp X-rays	DMF 2.40	[422]
WR-2721	400mg/kg 35-45min before radiation	female mouse skin	240kVp X-rays	DMF 1.55(in air) DMF 1.17 (in O_2)	[427]

Compound	Dose	Biological Material	Radiation	Effect	Reference
WR-2721	400mg/kg 30min before radiation	mouse lung	240kVp X-rays	DMF (pneumonitis) 1.2-2.4 DMF (fibrosis) 1.5-1.6	[444]
WR-2721	400mg/kg variable time between drug and radiation	mouse skin	e^-; 20-60 Gy 13-17 Gy/min	DMF 1.1-1.3 (5min) DMF 1.7-2.1 (30min)	[443]
WR-2721	200mg/kg	rat hind limb	^{60}Co-γ; 20-80 Gy 1.31 Gy/min	DMF (late skin reaction) 1.5 DMF (muscle damage) 1.5-2.0	[453]
WR-2721	(1) 125mg/kg (2) 250mg/kg 15min before radiation	mouse strains	300kVp X-rays	(1) DMF 1.34-1.40 (2) DMF 1.63-1.87	[489]
WR-2822	195mg/kg	C57Bl/GJ mice	(1) 4 MeV X-rays 250 R/min (2) 0.9 MeV neutrons 55 R/min	(1) survival DMF 1.23 (2) survival DMF 1.51	[104]
WR-2823	200mg/kg	C57Bl/GJ mice	(1) 4 MeV X-rays 250 R/min (2) 0.9 MeV neutrons 55 R/min	(1) survival DMF 1.32 (2) survival DMF 1.21	[104]

Compound	Dose	Biological Material	Radiation	Effect	Reference
WR-77913	2200mg/kg	BALB/C mice	^{60}Co-γ; 200 R/min	hematopoietic death DMF 1.97	[104]
	1500mg/kg	BALB/C mice	^{60}Co-γ; 200 R/min	gastrointestinal death DMF 1.95	
WR-109342	16.5mg/kg	C57Bl/6J mice	(1) 4 MeV X-rays 250 R/min (2) 0.9 MeV neutrons 55 R/min	(1) survival DMF 1.17 (2) survival DMF 1.46	[104]

ABBREVIATIONS USED IN COMPOUND LIST

P-Air, P-N_2 = ratio of doses unprotected to give 10% survival S. flexneri added to solution 5-10 min before radiation.

DMF - Dose Multiplying Factor: The ratio of radiation doses required in the presence or absence of the drug to achieve the same level of effect.

DRF - Dose Reduction Factor: Synonymous with DMF, but specific for radioprotection.

D_o - Slope of the cell survival curve.

i.m - intramuscular injection of drug.

i.p - intraperitoneal injection of drug.

kV_p - kilovolt peak: a measure of the intensity of X-rays.

ma - milliamperes.

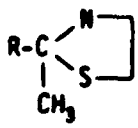
keV - kilo electron volts.

Gy - Gray, a unit of incident radiation (equal to 100 rads).

MeV - Million electron volts.

Structures: 1 = $HC{\equiv}C\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}SH$

2 & 3:



R = $CH_2{=}CH\text{-}CH_2\text{-}CH_2\text{-}$ (Structure 2)

$CH_2{=}C(CH_3)\text{-}CH_2\text{-}CH_2\text{-}$ (Structure 3)

VIII. References.

1. Abe M ; Nishidai T ; Yukawa Y ; Takahashi M ; Ono K ; Hiraoka M ; Ri N. Studies on the radioprotective effects of superoxide dismutase in mice. Int. J. Radiat. Oncol. Biol. Phys. 1981 7, 205-209.
2. Acosta D ; Sorenson EMB. Role of calcium in cytotoxic injury of cultured hepatocytes. Ann. N.Y. Acad. Sci. 1983 407, 78-92.
3. Adams GE. Hypoxia-mediated drugs for radiation and chemotherapy. Cancer 1981 48, 696-707.
4. Adams GE. Radiation chemical mechanisms in radiation biology. Adv. Radiat. Chem. 1972 3, 125-208.
5. Adams GE ; Armstrong RC ; Charlesby A ; Michael BD ; Willson RL. Pulse radiolysis of sulphur compounds. Part 3. Repair by hydrogen transfer of a macromolecule irradiated in aqueous solution. J. Chem. Soc. Transac. Faraday 1969 555, Part 3, 732-742.
6. Adams GE ; McNaughton GS ; Michael BD. Pulse radiolysis of sulphur compounds. Part 2. Free radical "repair" by hydrogen transfer from sulphydryl compounds. J. Chem. Soc. Transac. Faraday 1968 544, Part 4, 902-910.
7. Adams GE ; McNaughton GS ; Michael BD. The pulse radiolysis of sulphur compounds. Part I. Cysteamine and cystamine. In: "The Chemistry of Ionization and Excitation," (Proc. Conf. on Radiation Chemistry and Photochemistry, Univ. of Newcastle upon Tyne, 21-23 Sept., 1966) (GRA Johnson ; G Scholes, eds.) 1967, pp.281-293, Taylor and Francis, London.
8. Adams GE ; Michael BD ; Asquith JC ; Shenoy MA ; Watts ME ; Whillans DW. Rapid-mixing studies on the time-scale of radiation damage in cells. In: "Radiation Research: Biomedical, Chemical and Physical Perspectives," (OF Nygaard ; HI Adler ; WK Sinclair, eds.) 1975, pp.478-492. Academic Press, London.
9. Akerboom TPM ; Bilzer M ; Sies H. Competition between transport of glutathione disulfide (GSSG) and glutathione S-conjugates from perfused rat liver into bile. F.E.B.S.Lett. 1982 140, 73-76.
10. Akoyev IG. Problems of postradial recovery (Problemy postluchevogo vosstano-vieniva). 1975. Available through NTIS AD-A028 273/1.
11. Alexander P ; Bacq ZM ; Cousens SF ; Fox M ; Herve A ; Lazar J. Mode of action of some substances which protect against the lethal effects of X-rays. Radiat. Res. 1955 2, 392.
12. Alexander P ; Dean CJ ; Lehmann AR ; Ormerod MG ; Felshreiber P ; Serianni RW. The repair of DNA and the mode of action of sensitizers and protectors in biological systems of different complexity. In: "Radioprotection and Sensitization," (H Moroson ; M Quintiliani, eds.) 1970, pp.15-34. Taylor and Francis, London.
13. Alper T. Another method for testing the applicability of the hyperbolic oxygen equation and for evaluating K. Int. J. Radiat. Biol. 1976 30, 389-392.
14. Alper T. "Cellular Radiobiology," 1979, 320pp. Cambridge Univ. Press, Cambridge.
15. Alper T. Chemical protection of various bacteria and its involvement with the oxygen effect. Reported by EA Wright. In: "Radiation Effects in Physics, Chemistry and Biology," (M Ebert ; A Howard, ed.) 1963, pp.276-289. North Holland Publ. Co., Amsterdam.

16. Alper T. Lethal mutations and cell death. Phys. Med. Biol. 1963 8, 365-385.
17. Alper T. Observations on bacterial growth and morphology shortly after irradiation and some remarks on the oxygen effect. In: "Advances in Radiobiology," (GC DeHevesy ; AR Gunner ; JD Abbatt, eds.) 1956, pp.90-102. Oliver and Boyd, Edinburgh.
18. Alper T. Oxygen as radiosensitizer: Methods of analysis. Int. J. Radiat. Biol. 1983 44, 313-314.
19. Alper T. The modification of damage caused by primary ionization of biological targets. Radiat. Res. 1956 5, 573-586.
20. Alper T ; Bewley DK ; Fowler JF. Chemical protection against α-particle irradiation. Nature 1962 194, 1245-1247.
21. Alper T ; Cramp WA ; Hornsey S ; Orr JS ; Wheldon TE (eds.). "Cellular Repair of Radiation Damage: Mechanisms and Modifying Agents," 1984, Br. J. Cancer, v.49, Suppl.6, 317pp.
22. Alper T ; Howard-Flanders P. Role of oxygen in modifying the radiosensitivity of E. coli B. Nature 1956 178, 978-979.
23. Alper T ; Moore JL ; Bewley DK. LET as a determinant of bacterial radiosensitivity, and its modification by anoxia and glycerol. Radiat. Res. 1967 32, 277-293.
24. Ames BN ; Cathcart R ; Schwiers E ; Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis. Proc. Natl. Acad. Sci. U.S.A. 1981 78, 6858-6862.
25. Ames SR ; Elvehjem CA. Enzymatic oxidation of glutathione. J. Biol. Chem. 1945 159, 549-562.
26. Anbar M. Water and aqueous solutions. In: "Fundamental Processes in Radiation Chemistry," (P Ausloos, ed.) 1968, pp.651-685. Wiley-Interscience, New York.
27. Anbar M ; Bambenek M ; Ross AB. Selected specific rates of reactions of transients from water in aqueous solution. I. Hydrated electron. Natl. Stand. Ref. Data Ser., Natl. Bur. Stand. 1973. Available through NTIS COM-73-50537.
28. Anbar M ; Farhataziz R ; Ross AB. Selected specific rates of reactions of transients from water in aqueous solution. II. Hydrogen atom. Natl. Stand. Ref. Data Ser., Natl. Bur. Stand. 1975. Available through NTIS COM-75-10617/9GA.
29. Anderson RS ; Turkowitz H. The experimental modification of the sensitivity of yeast to roentgen rays. Am. J. Roentgenol. 1941 46, 537-541.
30. Ashwood-Smith MJ. Radioprotection and cryoprotective properties of dimethyl sulfoxide in cellular systems. Ann. N.Y. Acad. Sci. 1967 141, 45-62.
31. Ashwood-Smith MJ. The radioprotective action of dimethyl sulphoxide and various other sulphoxides. Int. J. Radiat. Biol. 1961 3, 41-48.
32. Aslamova LI ; Blyum YB ; Tsudzevitch BA ; Kucherenko NE. Study of DNA synthesis during irradiation and its protection by serotonin in the liver of rats after removal of the cycloheximide block. Radiobiologiya 1983 23, 157-160.
33. Asmus K-D. Sulfur-centered free radicals. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.23-42. Academic Press, New York.
34. Augustinsson K-B ; Jonsson S ; Sperman B. Effects of ionizing radiation on arylesterase and cholinesterase. Acta Chem. Scand. 1961 15, 11-15.

35. Bachur NR ; Gordon SL ; Gee MV. A general mechanism for microsomal activation of quinone anticancer agents to free radicals. Cancer Res. 1978 38, 1745-1750.
36. Bacq ZM. Chemical protection against ionizing radiations in mammals. Bull. Acad. R. Med. Belg. 1966 6, 115-141.
37. Bacq ZM. Recent research on the chemical protectors and particularly on cysteamine-cystamine. In: "Advances in Radiobiology," (GC DeHevesy ; AR Gunner ; JD Abbatt, eds.) 1956, pp.160-169. Oliver and Boyd, Edinburgh.
38. Bacq ZM ; Alexander P (eds.). "Fundamentals of Radiobiology," 1961, 2nd edition, 555pp. Pergamon Press, Oxford.
39. Bacq ZM ; Beaumariage ML ; Liebecq-Hutter S. Relation entre la radioprotection et l'hypothermie induite par certaines substances chimiques. Int. J. Radiat. Biol. 1965 9, 175-178.
40. Bacq ZM ; Beaumariage ML ; Van Caneghem P ; Ciccarone P. Importance of the pharmacological and biochemical action of cysteamine and related substances for their radioprotective effect in mammals. Ann. Ist. Super. Sanita 1965 1, 639-645.
41. Bacq ZM ; Herve A. Protection chimique contre le rayonnement X. Bull. Acad. Roy. Med. Belg. 1952 17, 13.
42. Bacq ZM ; Van Caneghem P. The shock produced by large doses of radioprotective SH or SS substances. Radiat Damage, Proc Panel, 1960, 141-147. IAEA PL-311/14.
43. Bahnemann D ; Asmus K-D ; Willson RL. Free radical reactions of the phenothizine metiazinic acid. J. Chem. Soc. Perkin Trans. II 1981, 890-895.
44. Baker MZ ; Badiello R ; Tamba M ; Quintiliani M ; Gorin G. Pulse radiolytic study of hydrogen transfer from glutathione to organic radicals. Int. J. Radiat. Biol. 1982 41, 595-602.
45. Baker WK. The oxygen effect and the mutation process. Brookhaven Symposia in Biology 1956 No.8, 191-200.
46. Barendsen GW ; Koot CJ ; Van Kersen GR ; Bewley DK ; Field SB ; Parnell CJ. The effect of oxygen on impairment of the proliferative capacity of human cells in culture by ionizing radiations of different LET. Int. J. Radiat. Biol. 1966 10, 317-327.
47. Barron ESG. The effect of ionizing radiations on systems of biological importance. Ann. N.Y. Acad. Sci. 1955 59, 574-594.
48. Barton JP ; Packer JE. The radiolysis of oxygenated cysteine solutions at neutral pH. The role of RSSR$^-$ and O_2^-. Int J. Radiat. Phys. Chem. 1970 2, 159-166.
49. Bartosz G ; Leyko W. Radioprotection of bovine erythrocytes to hemolysis. Int. J. Radiat. Biol. 1981 39, 39-46.
50. Bartosz G ; Leyko W ; Fried R. Is superoxide dismutase a physiological radioprotector? Experientia 1979 35, 1194.
51. Bartosz G ; Leyko W ; Kedziora J ; Jeske J. Superoxide dismutase and radiation-induced haemolysis: No benefit of its increased content in red cells. Int. J. Radiat. Biol. 1980 38, 187-192.
52. Beatty P ; Reed DJ. Influence of cysteine upon the glutathione status of isolated rat hepatocytes. Biochem. Pharmacol. 1981 30, 1227-1230.
53. Beatty P ; Reed DJ. Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. Arch. Biochem. Biophys. 1980 204, 80-87.
54. Behling UH ; Phuc Ung Pham ; Madani F ; Nowotny A. Components of lipopolysaccharide which induce colony stimulation, adjuvancy, and radioprotection. Microbiology (Washington D.C.) 1980, 103-107.

55. Belli JA ; Bonte FJ. Influence of temperature on the radiation response of mammalian cells in tissue culture. Radiat. Res. 1963 18, 272-276.
56. Belli JA ; Shelton M. Potentially lethal radiation damage: Repair by mammalian cells in culture. Science 1969 165, 490-492.
57. Belyakova NV ; Kravetskaya TP ; Krutyakov VM. Effect of radioprotectants, 2-mercaptoethylamine and 5-methoxytryptamine, on activity of some repair enzymes. Radiobiologiya 1981 21, 198-203.
58. Berdel WE ; Schick P ; Sedlmeier H ; Fink U ; Rastetter J ; Messerschmidt O. Experimental chemotherapy of radiation injury with synthetic lysophospholipid analogs in mice. Radiat. Res. 1983 94, 166-170.
59. Berteaud A-J. Interactions of electromagnetic fields with living cells and molecular systems. In: "Biological Effects and Dosimetry of Nonionizing Radiation - Radiofrequency and Microwave Energies," (M Grandolfo ; SM Michaelson ; A Rindi, eds.) 1983, pp.319-335. NATO Scientific Affairs Division. Plenum Press, New York.
60. Betz EH ; Mewissen DJ ; Closon J. Influence de la chlorpromazine sur la survie des rats irradies. Arch. Int. Pharmacodyn. Ther. 1959 121, 134-145.
61. Biaglow JE. Cellular electron transfer and radical mechanisms for drug metabolism. Radiat. Res. 1981 86, 212-242.
62. Bianchi V ; Zantedeschi A ; Ronchese F ; Levis AG. Induction of DNA repair synthesis by ultraviolet radiation and methylmethanesulphonate in cultured mouse lymphocytes. Cancer Lett. 1983 18, 21-27.
63. Bick YAE ; Brown JK. Protection after x-irradiation by 1,4-dithiothreitol of two mammalian cell types in vitro. Cytobios 1979 25, 163-174.
64. Bielski BHJ ; Gebicki JM. Species in irradiated oxygenated water. Adv. Radiat. Chem. 1970 2, 177-279.
65. Bielski BHJ ; Richter HW. Study of superoxide radical chemistry by stopped-flow radiolysis and radiation-induced oxygen-consumption. J. Am. Chem. Soc. 1977 99, 3019-3023.
66. Billen D. The effects of radioprotectors on DNA polymerase I directed repair synthesis and DNA strand breaks in toluene treated and x-irradiated Escherichia coli. Radiat. Res. 1983 95, 158-164.
67. Bird RP. Cysteamine as a protective agent with high-LET radiations. Radiat. Res. 1980 82, 290-296.
68. Blok J ; Loman H. The effects of γ-radiation in DNA. Curr. Top. Radiat. Res. 1973 9, 165-245.
69. Blumberg AL ; Nelson DF ; Gramkowski M ; Glover D ; Glick JH ; Yuhas JM ; Kligerman MM. Clinical trials of WR-2721 with radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 561-563.
70. Bond VP ; Friedmer TM ; Cronkite EP. Evaluation and management of the heavily irradiated individual. J. Nucl. Med. 1960 1, 221-238.
71. Bonifacic M ; Schafer K ; Mockel HJ ; Asmus K-D. Primary steps in the reactions of organic disulfides with hydroxyl radicals in aqueous solutions. J. Phys. Chem. 1975 79, 1496-1502.
72. Borek C. Nutritional, hormonal, and enzymatic factors as modulators of radiation and chemical onocogenesis in vitro. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.495-513. Academic Press, New York.
73. Borsa M ; Abraham AD ; Uray Z. Radioprotective effects of mediol and leucotrofina studied by the dynamic changes of some metabolic processes in the thymus and the liver of x-irradiated rats. Rev. Roum. Biol. Ser. Biol. Anim. 1981 26, 63-67.

74. Boyd SC ; Sesame HA ; Boyd MR. High concentrations of glutathione in glandular stomach: Possible implications for carcinogenesis. Science 1979 205, 1010-1012.
75. Braams R. A mechanism for the direct action of ionizing radiations. Nature 1963 200, 752-754.
76. Braams R. Changes in the radiation sensitivity of some enzymes and the possibility of protection against the direct action of ionizing particles. Radiat. Res. 1960 12, 113-119.
77. Braun K ; Fridovich I. Superoxide radical and superoxide dismutase: Threat and defense. Acta Physiol. Scand. Suppl. 1980 492, 9-18.
78. Bresler SE ; Noskin LA ; Stepanova IN ; Kuzovleva MA. Mechanism of radioprotection of chemical compounds on Escherichia coli cells. Mol. Gen. Genet. 1978 163, 75-85.
79. Bridges BA. Effect of chemical modifiers on inactivation and mutation-induction by γ-radiation in Escherichia coli J. Gen. Microbiol. 1963 31, 405-412.
80. Bridges BA. The chemical protection of Pseudomonas species against ionizing radiation. Radiat. Res. 1962 17, 801-808.
81. Brigelius R ; Muckel C ; Akerboom TPM ; Sies H. Identification and quantitation of glutathione in hepatic protein mixed disulfides and its relationship to glutathione disulfide. Biochem. Pharmacol. 1983 32, 2529-2534.
82. Broch H ; Cabrol D ; Vasilescu D. Quantum mechanical simulation of the interaction between the radioprotector cysteamine and DNA. Int. J. Quantum Chem., Quantum Biol. Symp. 1980 7, 283-295.
83. Brock N ; Pohl J. The development of Mesna for regional detoxification. Cancer Treat. Rev. 1983 10 Suppl.A, 33-43.
84. Brodie AE ; Potter J ; Reed DJ. Unique characteristics of rat spleen lymphocyte L1210 lymphoma and HeLa cells in glutathione biosynthesis from sulfur-containing amino acids. Eur. J. Biochem. 1982 123, 159-164.
85. Brown MR ; Fisher LA ; Sawchenko PE ; Swanson LW ; Vale WW. Biological effects of cysteamine: Relationship to somatostatin depletion. Regul. Peptides 1983 5, 163-179.
86. Brown WE. Mechanism of action of aminothiol radioprotectors. Nature 1967 213, 363.
87. Bryant PE. Survival after fractionated doses of radiation: Modification by anoxia of the response of Chlamydomonas. Nature 1968 219, 75-77.
88. Bucher JR ; Tien M ; Morehouse LA ; Aust SD. Redox cycling and lipid peroxidation: The central role of iron chelates. Fund. Appl. Toxicol. 1982 3, 222-226.
89. Bump EA ; Taylor YC ; Brown M. Role of glutathione in the hypoxic cell cytotoxicity of misonidazole. Cancer Res. 1983 43, 997-1002.
90. Burton GW ; Ingold KU. Autoxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J. Am. Chem. Soc. 1981 103, 6472-6477.
91. Butterworth BE ; Earle LL ; Strom S ; Jirtle R ; Michalopoulos G. Induction of DNA repair in human and rat hepatocytes by 1,6-dinitropyrene. Mutat. Res. 1983 122, 73-80.
92. Buxton GV. Basic radiation chemistry of liquid water. NATO Adv. Study Inst. Ser., Ser. C. (Study Fast Processes Transient Species Electron Pulse Radiolysis) 1982 86, 241-266.
93. Buxton GV. Nanosecond pulse radiolysis of aqueous solutions containing proton and hydroxyl radical scavengers. Proc. R. Soc. London, Ser. A. 1972 328, 9-21.

94. Cantoni O ; Costa M. Correlations of DNA strand breaks and their repair with cell survival following acute exposure to mercury(ii) and X-rays. Molec. Pharmacol. 1983 24, 84-89.
95. Capozzi G ; Modena G. Oxidation of thiols. In: "The Chemistry of the Thiol Group," (S Patai, ed.) 1974, Part 2, pp.785-839. John Wiley and Sons, New York.
96. Century B ; Horwitt M. Biological availability of various forms of vitamin E with respect to different indices of deficiency. Fed. Proc. 1965 24, 906-911.
97. Chan PC ; Bielski BHJ. Pulse radiolysis study of optical absorption and kinetic properties of dithiothreitol free radical. J. Amer. Chem. Soc. 1973 95, 5504-5508.
98. Chance B ; Sies H ; Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 1979 59, 527-605.
99. Chapman JD ; Reuvers AP ; Borsa J ; Greenstock CL. Chemical radioprotection and radiosensitization of mammalian cells growing in vitro. Radiat. Res. 1973 56, 291-306.
100. Chapman JD ; Sturrock J ; Boag JW ; Crookall JD. Factors affecting the oxygen tension around cells growing in plastic Petri dishes. Int. J. Radiat. Biol. 1970 17, 305-328.
101. Chenery R ; George M ; Krishna G. The effect of ionophore A23187 and calcium on carbon tetrachloride-induced toxicity in cultured rat hepatocytes. Toxicol. Appl. Pharmacol. 1981 60, 241-252.
102. Cho ES ; Sahyoun N ; Steglnk LD. Tissue glutathione as a cysteine reservoir during fasting and refeeding of rats. J. Nutr. 1981 111, 914-922.
103. Coleman CN ; Urtasun RC ; Wasserman TH ; Hancock S ; Harris JW ; Halsey J ; Hirst VK. Initial report of the Phase I trial of the hypoxic cell radiosensitizer SR 2508. In: "Chemical Modifiers of Cancer Treatment, Conference of 27 November - 1 December, Banff, Canada) 1984, pp.6-17.
104. Connor AM ; Sigdestad CP. Chemical protection against gastrointestinal radiation injury in mice by WR 2822, WR 2823, or WR 109342 after 4 MeV X-ray or fission neutron irradiation. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 547-551.
105. Coyle PJ ; Dainton FS ; Logan SR. The probable relaxation in time of the ionic atmosphere of the hydrated electron. Proc. Chem. Soc., London 1964, 219.
106. Cramp WA. Radiation protection of Shigella flexneri by compounds containing the thiourea molecular structure. In: "Energy Transfer in Radiation Processes," (GO Phillips, ed.) 1965, pp.153-159. Elsevier Press, Amsterdam.
107. Cramp WA. Radiation protection of Shigella flexneri by ethanol, β-mercaptoethanol and several polyhydric alcohols. Int. J. Radiat. Biol. 1969 15, 227-232.
108. Cramp WA ; Edwards JC ; George AM ; Sabovljev SA. Subcellular lesions: The current position. In: "Cellular Repair of Radiation Damage: Mechanisms and Modifying Agents," (T Alper ; WA Cramp ; S Hornsey ; JS Orr ; TE Wheldon, eds.) 1984, Br. J. Cancer, v.49, Suppl.6, pp. 7-12.
109. Cullen B ; Lansky I. The effect of pre-irradiation growth conditions on the relative radiosensitivities of mammalian cells at low oxygen concentrations. Int. J. Radiat. Biol. 1974 26, 579-588.
110. Cullen BM ; Walker MC. Variation of the radiobiological oxygen constant, K, with the proliferative activity of the cells. Int. J. Radiat. Biol. 1980 38, 513-524.

111. Czaplicki J ; Blonska B ; Stec L. The effect of embryonal thymic calf extracts on neonatally thymectomized mice and on mice lethally irradiated with γ-rays. Thymus 1981 3, 143-151.
112. Dale WM ; Gray LH ; Meredith WJ. The inactivation of an enzyme (carboxypeptidase) by x- and α-radiation. Trans. Royal Soc., London 1949 242, 33-62.
113. Defais MJ ; Hanawalt PC. Viral probes for DNA repair. Adv. Radiat. Biol. 1983 10, 1-37.
114. Deffippes H ; Guild WR. Irradiation of solutions of transforming DNA. Radiat. Res. 1959 11, 38-53.
115. Demple B ; Halbrook J. Inducible repair of oxidative DNA damage in Escherichia coli. Nature 1983 304, 466-468.
116. Denekamp J ; Michael BD ; Rojas A ; Stewart FA. Radioprotection of mouse skin by WR-2721: The critical influence of oxygen tension. Int. J. Radiat. Oncol. 1982 8, 531-534.
117. Denekamp J ; Michael BD ; Rojas A ; Stewart FA. Thiol radioprotection in vivo: The critical role of tissue oxygen concentration. Br. J. Radiol. 1981 54, 1112-1114.
118. Denekamp J ; Rojas A ; Stewart FA. Is radioprotection by WR-2721 restricted to normal tissues? In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.655-679. Academic Press, New York.
119. Denekamp J ; Stewart FA ; Rojas A. Is the outlook grey for WR-2721 as a clinical radioprotector? Int. J. Radiat. Oncol. Biol. Phys. 1983 9, 1247-1249.
120. Dertinger H ; Jung H. "Molecular Radiation Biology," 1970, 237pp. Springer Verlag, New York.
121. Deschavanne PJ ; Midlander J ; Edgren M ; Larsson A ; Malaise EP ; Revesz L. Oxygen enhancement of radiation induced lethality is greatly reduced in glutathione deficient human fibroblasts. Biomedicine 1981 35, 35-37.
122. Deschner EE ; Gray LH. Influence of oxygen tension on X-ray-induced chromosomal damage in Ehrlich ascites tumor cells irradiated in vitro and in vivo. Radiat. Res. 1959 11, 115-146.
123. Devik F ; Lothe F. The effect of cysteamine, cystamine and hypoxia on mortality and bone marrow chromosome aberrations in mice after total body roentgen irradiation. Acta Radiologica 1955 44, 243.
124. Dewey DL. The X-ray sensitivity of Serratia marcescens. Radiat. Res. 1963 19, 64-87.
125. Dewhurst HA ; Samuel AH ; Magee JL. A theoretical survey of the radiation chemistry of water and aqueous solutions. Radiat. Res. 1954 1, 62-83.
126. Dickens EA ; Shapiro B. Protein binding of AET and its alteration by ionizing radiation. U.S. Dept. Comm. Off. Tech. Serv., PB Rep. 146,190 1960, pp.11.
127. Dickens EA ; Shapiro B. The mechanism of action of AET. II. The interaction between proteins and 2-mercaptoethylguanidine and bis(2-guanidoethyl) disulfide in aqueous buffered solutions. Radiat. Res. 1961 14, 308-322.
128. Doherty DG ; Burnett WT Jr ; Shapira R. Chemical protection against ionizing radiation. II. Mercaptoalkylamines and related compounds with protective activity. Radiat. Res. 1957 7, 13-21.

129. Dorfman LM ; Taub IA. Pulse radiolysis studies. III. Elementary reactions in aqueous ethanol solution. J. Am. Chem. Soc. 1963 85, 2370-2374.
130. Draganic IG ; Draganic ZD. "The Radiation Chemistry of Water," 1971, 256pp. Academic Press, New York.
131. Du D ; Shi B ; Liu D ; Ren J. Radioprotective effect of superoxide dismutase. Zhonghua Fangshe Yixue Yu Fanghu Zazhi 1982 2, 26-30.
132. Durand RE. Radioprotection by WR-2721 in vitro at low oxygen tensions: Implications for its mechanisms of action. Br. J. Cancer 1983 47, 387-392.
133. Eberle D ; Clarke R ; Kaplowitz N. Rapid oxidation in vitro of endogenous and exogenous glutathione in bile of rats. J. Biol. Chem. 1981 256, 2115-2117.
134. Edelman IS ; Olney JM ; Brooks L ; Moore FD. Body composition: Studies in the human being by the dilution principle. Science 1952 115, 447-454.
135. Edgren M. Depletion of cellular thiols by misonidazole treatment prevents some post-irradiation repair processes. Br. J. Radiol. 1983 56, 211-212.
136. Edgren M. Intercellular co-operation in repairing radiation-induced single-strand DNA breaks. Int. J. Radiat. Biol. 1982 41, 589-593.
137. Edgren M ; Révész L ; Larsson A. Induction and repair of single-strand DNA breaks after X-irradiation of human fibroblasts deficient in glutathione. Int. J. Radiat. Biol. 1981 40, 355-363.
138. Edwards JC ; Chapman D ; Cramp WA. Radiation studies of Acholeplasma laidlawii: The role of membrane composition. Int. J. Radiat. Biol. 1983 44, 405-412.
139. Edwards JC ; Chapman D ; Cramp WA. The effects of ionizing radiation on the peroxide content of a pure polyunsaturated lipid dispersion and of lipids and membranes derived from Acholeplasma laidlawii. Int. J. Radiat. Biol. 1984 45, 33-44.
140. Elbers R ; Heldt HW ; Schmucker P ; Soboll S ; Wiese H. Measurement of the ATP/ADP ratio in mitochondria and in the extramitochondrial compartment by fractionation of freeze-stopped liver tissue in non-aqueous media. Hoppe-Seyler's Z. Physiol. Chem. 1974 355, 378-393.
141. Eldjarn L ; Pihl A. On the mode of action of X-ray protective agents. I. The fixation in vivo of cystamine and cysteamine to proteins. J. Biol. Chem. 1956 223, 341-352.
142. Eldjarn L ; Pihl A. On the mode of action of X-ray protective agents. II. Interaction between biologically important thiols and disulfides. J. Biol. Chem. 1956 225, 499-510.
143. Eldjarn L ; Pihl A. The cysteine-cysteamine group of protective agents: Chemical structure, protective ability, and mixed disulfide formation. Radiat. Res. 1958 9, 110.
144. Eldjarn L ; Pihl A. The equilibrium constants and oxidation-reduction potentials of some thiol-disulfide systems. J. Amer. Chem. Soc. 1957 79, 4589-4593.
145. Elkind MM ; Sutton H. Radiation response of mammalian cells grown in culture. I. Repair of X-ray damage in surviving Chinese hamster cells. Radiat. Res. 1960 13, 556-593.
146. Elkind MM ; Sutton H. X-ray damage and recovery in mammalian cells in culture. Nature 1959 184, 1293-1295.
147. Emerit I ; Cerutti P. Clastogenic activity from Bloom syndrome fibroblast cultures. Proc. Natl. Acad. Sci. U.S.A. 1981 78, 1868-1872.

148. Emerit I ; Keck M ; Levy A ; Feingold J ; Michelson M. Activated oxygen species at the origin of chromosome breakage and sister chromatid exchanges. Mutat. Res. 1982 103, 165-172.
149. Epp ER ; Kessaris ND ; Santomasso A ; Heslin J ; Ling CC. Oxygen diffusion times in bacterial cells irradiated with high intensity pulsed electrons: New upper limit to the lifetime of oxygen sensitive species suspected to be induced at critical sites in bacterial cells. Radiat. Res. 1973 54, 171-180.
150. Epp ER ; Weiss H ; Ling CC. Irradiation of cells by single and double pulses of high intensity radiation: Oxygen sensitization and diffusion kinetics. Curr. Top. in Radiat. Res. Q. 1976 11, 201-250.
151. Evans JW ; Taylor YC ; Brown JM. The role of glutathione and DNA strand break repair in determining the shoulder of the radiation survival curve. Br. J. Cancer 1984 49, Suppl.6, 49-53.
152. Ewing D ; Powers EL. Irradiation of bacterial spores in water: Three classes of oxygen dependent damage. Science 1976 194, 1049-1051.
153. Farhataziz R ; Ross AB. Selected specific rates of reactions of transients from water in aqueous solution. III. Hydroxyl radical and perhydroxyl radical and their ions. Natl. Stand. Ref. Data Series Natl. Bur. Stand. 1977, 59 113pp. (NBS Catalog No. C13-48:59) GPO, Wash DC 122pp. Available through NTIS PB-263198.
154. Ferle-Vidovic A ; Petrovic D ; Osmak M ; Kadija K. Radioprotection against fast neutrons. Radiol. Iugosl. 1982 16, 337-340.
155. Ferle-Vidovic A ; Petrovic D ; Vidic Z ; Osmak M ; Kadija K. Absence of 2-aminoethyl-isothiuronium bromide hydrobromide protection against fast neutrons cellular effects. Radiat. Environ. Biophys. 1981 19, 197-204.
156. Feuer L ; Benko G. Effect of glutaurine and its derivatives and their combinations with radiation protective substances on irradiated mice. Acta Radiol. Oncol. Radiat. Ther. Phys. Biol. 1981 20, 319-324.
157. Flohé L. Glutathione peroxidase brought into focus. In: "Free Radicals in Biology," (WA Pryor, ed.) 1981, v.5, pp.223-254. Academic Press, New York.
158. Fong K ; McCay PB ; Poyer JL ; Keele BB ; Misra H. Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. J. Biol. Chem. 1973 248, 7792-7797.
159. Forni LG ; Mönig J ; Mora-Arellano VO ; Willson RL. Thiyl free radicals: Direct observations of electron transfer reactions with phenothiazines and ascorbate. J. Chem. Soc. Perkin Trans. II 1983, 961-965.
160. Forni LG ; Willson RL. Vitamin C and consecutive hydrogen atom and electron transfer reactions in free radical protection: A novel catalytic role for glutathione. In: "Protective Agents in Cancer," (DCH McBrien ; TF Slater, eds.) 1983, pp.159-173. Academic Press, London.
161. Forsberg JO ; Hillered L ; Graffman S ; Jung B ; Persson E ; Selen G. Kidney radioprotection by temporary hypoxia. Experiments with degradable microspheres. Scand. J. Urol. Nephrol. 1981 15, 147-152.
162. Fowler JF. What next in fractionated radiotherapy? The first James Kirk Memorial Lecture. In: "Cellular Repair of Radiation Damage: Mechanisms and Modifying Agents," (T Alper ; WA Cramp ; S Hornsey ; JS Orr ; TE Wheldon, eds.) 1980, Br. J. Cancer, v.49, Suppl.6, pp. 285-300.
163. Foye WO. Radioprotective drugs. In: "Burger's Medicinal Chemistry," (ME Wolff, ed.) 1981, Part III, Chapt.37, pp.11-45. Wiley & Sons, New York.

164. Foye WO ; Karkaria MM ; Parsons WH. Antiradiation compounds. XVII. Binding ability of radiation-protective N-heterocyclic aminoethyl disulfides and thiosulfates to DNA. J. Pharm. Sci. 1980 69, 84-87.
165. Fridovich I. I. Oxygen radicals, hydrogen peroxide, and oxygen toxicity. In: "Free Radicals in Biology," (WA Pryor, ed.) 1976, v.1, pp.239-277. Academic Press, New York.
166. Friedberg EC ; Ehmann UK ; Williams JI. Human diseases associated with defective DNA repair. Adv. Radiat. Biol. 1979 8, 85-174.
167. Galutsov B ; Todorov S ; Ivanov S ; Marinoposlki G. Effect of AET and adeturon on mitochondrial protein synthesis. Radiobiologiya 1980 20, 334-337.
168. Garriott ML ; Crowe DT. AET reduces the frequency of micronuclei in bone marrow cells of mice exposed to γ-radiation. Radiat. Res. 1983 93, 200-204.
169. Geard CR. Effects of radiation on chromosomes. In "Radiation Biology," (DJ Pizzarello, ed.) 1982, pp.83-109. CRC Press, Inc., Boca Raton, Florida.
170. Gennaro R ; Romeo D. The release of superoxide anion from granulocytes: Effect of inhibitors of anion permeability. Biochem. Biophys. Res. Commun. 1979 88, 44-99.
171. Gerstenecker C ; Sies H. Restriction of hexobarbital metabolism by t-butyl hydroperoxide in perfused rat liver. Biochem. Pharmacol. 1980 29, 3112-3113.
172. Ginoza W ; Norman A. Radiosensitive molecular weight of tobacco mosaic virus nucleic acid. Nature 1957 179, 520-521.
173. Glatt H ; Protic-Sabljic M ; Oesch F. Mutagenicity of glutathione and cysteine in the Ames test. Science 1982 220, 961-963.
174. Glover D ; Riley L ; Carmichael K ; Spar B ; Glick J ; Kligeman MM ; Agus ZS ; Slatopolsky E ; Attie M ; Goldfarb S. Hypocalcemia and inhibition of parathyroid hormone secretion after administration of WR-2721 (a radioprotective and chemoprotective agent). New Engl. J. Med. 1983 309, 1137-1141.
175. Gordon S ; Hart EJ ; Matheson MS ; Rabani J ; Thomas JK. Reaction constants of the hydrated electron. J. Am. Chem. Soc. 1963 85, 1375-1377.
176. Gorin G. Mercaptan-disulfide interchange and radioprotection. Prog. Biochem. Pharmacol. 1965 1, 142-151.
177. Gorin G ; Quintiliani M. Radiation damage in some enzymes and their chemical protection. Radiat. Res. 1965 25, 193.
178. Graevskii E Ya ; Konstantinova MM. Independence of the radiation-protective action of aminoethylisothiuronium bromide hydrobromide from the oxygen effect. Dokl. Akad. Nauk. SSSR 1961 140, 705-708.
179. Graevskii E Ya ; Konstantinova MM ; Nekrasova IV ; Sokolova OM ; Tarasenko AG. Correlation between changes in radiation sensitivity and level of endogenous thiol groups under the effect of radioprotective agents. Radiobiologiya 1967 7, 130-132.
180. Graevskii E Ya ; Yanushevskaya MI ; Bueverova EI ; Bragina EV ; Konstantinova MM. Investigation of radioprotective efficacy of biogenous amines and some aspects of the mechanism of their action on mammalian cells cultivated in vitro. Radiobiologiya 1981 21, 683-687.
181. Grandolfo M ; Michaelson SM ; Rindi A (eds.). "Biological Effects and Dosimetry of Nonionizing Radiation - Radiofrequency and Microwave Energies," 1983, 669pp. NATO Scientific Affairs Division. Plenum Press, New York.

182. Gray JL ; Tew JT ; Jensen H. Protective effect of serotonin and of para-aminopropiophenone against lethal doses of x-radiation. Proc. Soc. Exp. Biol. Med. 1952 80, 604-607.
183. Gray LH. A method of oxygen assay applied to a study of the removal of dissolved oxygen by cysteine and cysteamine. In: "Progress in Radiobiology," (JS Mitchell ; BE Holmes ; CL Smith, eds.) 1956, pp.267-274, Oliver & Boyd, Edinburgh.
184. Griffith OW ; Meister A. Glutathione: Interorgan translocation, turnover and metabolism. Proc. Natl. Acad. Sci. U.S.A. 1979 76, 5606-5610.
185. Grundler W. Biological effects of radiofrequency and microwave energy at the molecular and cellular level. In: "Biological Effects and Dosimetry of Nonionizing Radiation - Radiofrequency and Microwave Energies," (M Grandolfo ; SM Michaelson ; A Rindi, eds.) 1983, pp.299-318. NATO Scientific Affairs Division. Plenum Press, New York.
186. Grzelinska E ; Bartkowiak A ; Bartosz G ; Leyko W. Effect of hydroxyl radical scavengers on radiation damage to the erythrocyte membrane. Int. J. Radiat. Biol. 1982 41, 473-482.
187. Gupta ML ; Singh RP ; Devi PU. Protection of mouse liver by 2-mercaptopropionylglycine against β-radiations from injected tritiated water. J. Radiat. Res. 1979 20, 329-337.
188. Haenen GMM ; Bast A. Protection against lipid peroxidation by a microsomal glutathione dependent labile factor. F.E.B.S. Lett. 1983 159, 24-28.
189. Hagan MP ; Riklis E. Aminoethanethiol effects on cell killing after BrdUrd near-UV treatment. Radiat. Res. 1982 91, 375.
190. Hall, JD ; Mount DW. Mechanisms of DNA replication and mutagenesis in ultraviolet-irradiated bacterial and mammalian cells. Prog. in Nucl. Acid Res. 1981 26, 53-126.
191. Hanawalt PC ; Cooper PK ; Ganesan AK ; Smith CA. DNA repair in bacteria and mammalian cells. Ann. Rev. Biochem. 1979 48, 783-836.
192. Harman LS ; Mottley C ; Mason RP. Free radical metabolites of L-cysteine oxidation. J. Biol. Chem. 1984 259, 5606-5611.
193. Hart EJ ; Anbar M. "The Hydrated Electron," 1970, 267pp. Wiley-Interscience, New York.
194. Hart EJ ; Fielden EM. Submicromolar analysis of hydrated electron scavengers. Adv. Chem. 1965 50, 253-262.
195. Held KD ; Powers EL. Effects of varying O_2 concentration on the X-ray sensitivity of transforming DNA. Int. J. Radiat. Biol. 1979 36, 613-619.
196. Henle KJ ; Dethlefsen LA. Heat fractionation and thermotolerance: A review. Cancer Res. 1978 38, 1843-1851.
197. Henle KJ ; Nagle WA ; Moss AJ Jr. Development of thermotolerance following the oxidation of cellular glutathione. Radiat. Res. 1983 94, 584.
198. Henriksen T. Effect of the irradiation temperature on the production of free radicals in solid biological compounds exposed to various ionizing radiations. Radiat. Res. 1966 27, 694-709.
199. Higashi T ; Tateishi N ; Naruse A ; Sakamoto Y. A novel physiological role of liver glutathione as a reservoir of L-cysteine. J. Biochem. 1977 82, 117-124.
200. Hill KE ; Burk RF. Effect of selenium deficiency and vitamin E deficiency on glutathione metabolism in isolated rat hepatocytes. J. Biol. Chem. 1982 257, 10668-10672.

201. Hill KE ; Burk RF. Influence of vitamin E and selenium on glutathione-dependent protection against microsomal lipid peroxidation. Biochem. Pharmacol. 1984 33, 1065-1068.
202. Hill RP ; Porter LS ; Ives SA ; Wong T-Z. Initial studies of hypoxic radioprotection by deoxygenated dextran-hemoglobin. Int. J. Radiat. Oncol. Biol. Phys. 1984 10, 369-373.
203. Hiller K-O ; Masloch B ; Göbl M ; Asmus K-D. Mechanism of the OH· radical induced oxidation of methionine in aqueous solution. J. Am. Chem. Soc.1981 103, 2734-2743.
204. Hoffman MZ ; Hayon E. Pulse radiolysis study of sulfhydryl compounds in aqueous solution. J. Phys. Chem. 1973 77, 990-996.
205. Hollander A ; Doudney CO. Studies on the mechanism of radiation protection and recovery with cysteamine and β-mercaptoethanol. Radiobiology Symposium 1954, pp.112-115.
206. Holmes B. Inactivation of ribonuclease in dilute aqueous solutions. Nature 1950 166, 266-267.
207. Holt JA. The principles of hyperbaric and anoxic radiotherapy. Br. J. Radiol. 1975 48, 819-826.
208. Holthusen H. Beitrage zur Biologie der Strahlenwirkung. Untersuchungen an Askarideneiern. Pflügers Arch Gesamte Physiol. 1921 187, 1-24.
209. Hope DB. Radio-protective substances and hypothermia. Br. J. Radiol. 1958 31, 339.
210. Howard-Flanders P. Effect of oxygen on the radiosensitivity of bacteriophage in the presence of sulphydryl compounds. Nature 1960 186, 485-487.
211. Howard-Flanders P ; Alper T. The sensitivity of microorganisms to irradiation under controlled gas conditions. Radiat. Res. 1957 7, 518-540.
212. Howard-Flanders P ; Levin J ; Theriot L. Reactions of deoxyribonucleic acid radicals with sulfhydryl compounds in x-irradiated bacteriophage systems. Radiat. Res. 1963 18, 593-606.
213. Howard-Flanders P ; Moore D. The time interval after pulsed irradiation within which injury to bacteria can be modified by dissolved oxygen. Radiat. Res. 1958 9, 422-437.
214. Hunter N ; Milas L. Protection by S-2-(3-aminopropylamino)ethylphosphorothioic acid against radiation-induced leg contractures in mice. Cancer Res. 1983 43, 1630-1632.
215. Hutchinson F. Sulfhydryl groups and the oxygen effect on irradiated dilute solutions of enzymes and nucleic acid. Radiat. Res. 1961 14, 721-731.
216. Hütterman J ; Köhnlein W ; Teoule R (eds.). "Effects of Ionizing Radiation on DNA. Physical, Chemical and Biological Aspects," 1978, 383pp. Springer-Verlag, Berlin.
217. Idell-Wenger JA ; Grotyohann LW ; Neely JR. Coenzyme A and carnitine distribution in normal and ischemic hearts. J. Biol. Chem. 1978 253, 4310-4318.
218. Iliakis G ; Nüsse M ; Bryant P. Effects of aphidicolin on cell proliferation, repair of potentially lethal damage and repair of DNA strand breaks in Ehrlich ascites tumour cells exposed to X-rays. Int. J. Radiat. Biol. 1982 42, 417-434.
219. Iyanagi T ; Yamazaki I. One-electron-transfer reactions in biochemical systems. V. Difference in the mechanism of quinone reduction by the NADH dehydrogenase and the NAD(P)H dehydrogenase (DT-diaphorase). Biochim. Biophys. Acta 1970 216, 282-294.

220. Jamieson D ; Van den Brenk HAS. Studies of mechanisms of chemical radiation protection in vivo. III. Changes in fluorescence of intracellular pyridine nucleotides and modification by extracellular hypoxia. Int. J. Radiat. Biol. 1966 10, 223-241.
221. Jellum E. Interaction of cystamine and cystamine derivatives with nucleic acids and nucleophiles. Int. J. Radiat. Biol. 1965 9, 185-200.
222. Jellum E. The role of cystamine-nucleic-acid interactions in protection against X-ray-induced damage of DNA. Int. J. Radiat. Biol. 1966 10, 577-594.
223. Jocelyn PC. "Biochemistry of the SH Group," 1972, 404pp. Academic Press, London.
224. Jocelyn PC. Some properties of mitochondrial glutathione. Biochim. Biophys. Acta 1975 396, 427-436.
225. Jocelyn PC ; Kamminga A. The non-protein thiol of rat liver mitochondria. Biochim. Biophys. Acta 1974 343, 356-362.
226. Johnston D ; Oppermann H ; Jackson J ; Levinson W. Induction of four proteins in chick embryo cells by sodium arsenite. J. Biol. Chem. 1980 255, 6975-6980.
227. Jonah CD ; Matheson MS ; Miller JR ; Hart EJ. Yield and decay of the hydrated electron from 100 ps to 3 ns. J. Phys. Chem. 1976 80, 1267-1270.
228. Jonah CD ; Miller JR. Yield and decay of the OH radical from 200 ps to 3 ns. J. Phys. Chem. 1977 81, 1974-1976.
229. Jozwiak Z. Radiation damage to the erythrocyte membrane in the presence of radical anions. Int. J. Radiat. Biol. 1983 43, 195-200.
230. Jozwiak Z ; Helszer Z. Participation of free oxygen radicals in damage of porcine erythrocytes. Radiat. Res. 1981 88, 11-19.
231. Kada T ; Inoue T ; Yokoiyama A ; Mochizuki H ; Nakatsugawa S ; Sugahara T. Repair of DNA damage induced by ionizing radiation in Ataxia telangiectasia cells and potentially lethal damage repair inhibitors. In: "Modification of Radiosensitivity in Cancer Treatment," (T Sugahara, ed.) 1984, Chapt.14, pp.252-263. Academic Press Japan, Tokyo.
232. Kaiser F. Theory of resonant effects of radiofrequency and microwave energy. In: "Biological Effects and Dosimetry of Nonionizing Radiation - Radiofrequency and Microwave Energies," (M Grandolfo ; SM Michaelson ; A Rindi, eds.) 1983, pp.251-282. NATO Scientific Affairs Division. Plenum Press, New York.
233. Kao H-T ; Nevins JR. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. Molec. Cellul. Biol. 1983 3, 2058-2065.
234. Kapp DS ; Hahn GM. Thermosensitization by sulfhydryl compounds of exponentially growing Chinese hamster cells. Cancer Res. 1979 39, 4630-4635.
235. Kappus H ; Sies H. Toxic drug effects associated with oxygen metabolism: Redox cycling and lipid peroxidation. Experientia 1981 37, 1233-1240.
236. Kelly PM ; Schlesinger MI. The effect of amino acid analogues and heat shock on gene expression in chicken embryo fibroblasts. Cell 1978 15, 1277-1286.
237. Khare S ; Trivedi A ; Kesavan PC ; Prasad R. Effect of γ-radiation on the structure and function of yeast candida-albicans membrane. Int. J. Radiat. Biol. 1982 42, 369-384.

238. Kinnamon KE ; Ketterling LL ; Ledney GD ; Lorenz GB ; Mioduszewski RJ ; Stampfil HF. Survival of bone marrow-engrafted mice subsequent to protection from lethal radiation by WR 2721. Radiat. Res. 1980 82, 215-219.
239. Klayman DL ; Copeland ES. The design of antiradiation agents. In: "Drug Design," (EJ Ariens, ed.) 1975, v.6, Chapt.2, pp.82-142. Academic Press, New York.
240. Kligerman MM ; Blumberg AL ; Glick JH ; Nelson DF ; Glover D ; Yuhas JM ; Amols HI ; Goodman RL. Phase I trials of WR-2721 in combination with radiation therapy and with the alkylating agents cyclophosphamide and cis-platinum. Cancer Clin. Trials 1981 4, 469-474.
241. Klots CE. Energy deposition mechanisms. In: "Fundamental Processes in Radiation Chemistry," (P Ausloos, ed.) 1968, pp.1-57. Wiley-Interscience, New York.
242. Knox SJ ; Misra MP ; Shifrine M ; Roseblatt LS. Radiation induced inhibition of human lymphocyte blastogenesis, the effect of superoxide dismutase and catalase. Int. J. Radiat. Biol. 1982 41, 283-294.
243. Koch CJ. Measurement of very low oxygen tensions in liquids: Does the extrapolation number for mammalian survival curves decrease after x-irradiation under anoxic conditions? In: "Proc. 6th C.H. Gray Conf., 1974," (T Alper, ed.) 1975, pp.167-173. John Wiley & Sons, New York.
244. Koch CJ. Oxygen effects in radiobiology. Adv. Exp. Med. 1982 157, 123-144.
245. Koch CJ. The effect of oxygen on the repair of radiation damage by cells and tissues. Adv. Radiat. Biol. 1979 8, 273-315.
246. Koch CJ ; Stobbe CC ; Bump EA. The effect on the K_m for radiosensitization at 0°C of thiol depletion by diethylmaleate pretreatment: Quantitative differences found using the radiation sensitizing agent misonidazole or oxygen. Radiat. Res. 1984 98, 141-153.
247. Kollman G ; Castel N ; Shapiro B. Further studies on protection of DNA against ionizing radiation. Int. J. Radiat. Biol. 1970 18, 587-594.
248. Kollman G ; Shapiro B ; Leon S ; Martin D. The distribution and metabolism of the radiation protective agent aminopropylaminoethylphosphorothioate (WR-2721) in mice. 1978. Available through NTIS AD-A070 993/1.
249. Kollmann G ; Shapiro B ; Martin D. Mechanism of the protective action of GED against radiation damage to DNA. Radiat. Res. 1967 31, 721-731.
250. Kollman G ; Yuhas JM ; Leone S ; Shapiro B. Mechanism of differential radiation protection of tumor versus normal tissues by WR-2721 in tumor bearing mice. Radiat. Res. 1973 55, 603.
251. Konings AWT. Radiation protection of membranes by α-tocopherol. Int. J. Radiat. Biol. 1980 38, 119.
252. Konings AWT ; Oosterloo SK. Radiation effects on membranes. II. A comparison of the effects of X-irradiation and ozone exposure with respect to the relation of antioxidant concentration and the capacity for lipid peroxidation. Radiat. Res. 1980 81, 200-207.
253. Kosower NS ; Kosower EM. The glutathione status of cells. Int. Rev. Cytol. 1978 54, 109-160.
254. Krinsky NI ; Denecke SM. Interaction of oxygen and oxy-radicals with carotenoids. J. Natl. Cancer Inst. 1982 69, 205-210.
255. Krizala J ; Stoklasova A ; Kovarova H ; Ledvina M. Importance of superoxide dismutase in radiobiology. Biologia (Bratislava) 1983 38, 367-376.

256. Kuna P. Radioprotection of small intestine and spleen hemopoiesis by gammaphos (WR-2721) or cystamine in whole body γ-irradiated mice. Biologia (Bratislava) 1983 38, 273-282.
257. Kuna P. Radioprotective and hemodynamic action of cystamine given intramuscularly in rats. Radiobiol. Radiother. 1981 22, 793-800.
258. Kupperman A. Diffusion kinetics in radiation chemistry: An assessment. In: "Physical Mechanisms in Radiation Biology," (RD Cooper ; RW Wood, eds.) 1974, pp.115-176. Technical Information Center, United States Nuclear Regulatory Commission, Washington D.C.
259. Lakatos L ; Oroszlan G ; Dezsi Z ; Hatvani I ; Karmazsin L. Age-related difference in radioprotective effect of D-penicillamine. Dev. Pharmacol. Ther. 1982 5, 120-126.
260. Lal M. ^{60}Co γ-radiolysis of reduced glutathione in aerated solutions at pH values between 1 - 7.0. Can. J. Chem. 1976 54, 1092-1097.
261. Lampe FW ; Field FH ; Franklin JL. Reactions of gaseous ions. IV. Water. J. Am. Chem. Soc. 1957 79, 6132-6135.
262. Landolt R ; Arn D ; Cordt I ; Schaeppi K ; Fritz-Niggli H. Piotron pion irradiation of cerebral capillaries in neonatal rats. Relative biological effectiveness and radioprotection by O-(β-hydroxyethyl)rutosides. Radiat. Environ. Biophys. 1982 21, 75-80.
263. Landry J ; Bernier D ; Chrétien P ; Nicole LM ; Tanguay RM ; Marceau N. Synthesis and degradation of heat shock proteins during development and decay of thermotolerance. Cancer Res. 1982 42, 2457-2461.
264. Lanks KW. Metabolite regulation of heat shock protein levels. Proc. Natl. Acad. Sci. U.S.A. 1983 80, 5325-5329.
265. Larsson A. 5-Oxoprolinuria and other inborn errors related to the γ-glutamyl cycle. In: "Transport and Inhereted Disease," (MR Belton and C Toothill, eds.) 1981, pp.277-306. MTP Press, Boston.
266. Lea DEA. A theory of the actions of radiations on biological materials capable of recovery: Part I. The time-intensity factor. Br. J. Radiol. 1938 11, 489-498.
267. Lehnert S ; Fisher G ; Methot G. Radioprotection of normal and malignant tissue in the mouse by diethylaminoreserpine. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 1981 40, 63-74.
268. Leyko W ; Jozwiak Z ; Helszer Z ; Bartosz G. In vitro radioprotection of erythrocytes by superoxide dismutase. Dev. Biochem. (Biol. Clin. Aspects Superoxide Superoxide Dismutase) 1980 115, 311-317.
269. Li GC. Induction of thermotolerance and enhanced heat shock protein synthesis in Chinese hamster fibroblasts by sodium arsenite and by ethanol. J. Cellul. Physiol. 1983 115, 116-122.
270. Li GC ; Werb Z. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 1982 79, 3218-3222.
271. Li S ; Lu R. Radioprotective effect of 2,2-dimethylthiazolidine hydrochloride on hemopoietic stem cells. Zhonghua Fangshe Yixue Yu Fanghu Zazhi 1982 2, 26-28, 70-71.
272. Lindahl T. DNA glycosylases, endonucleases for apurinic/apyridinic sites, and base excision repair. Prog. Nucleic Acid Res. Mol. Biol. 1979 22, 135-192.
273. Ling CC ; Michaels HB ; Epp ER ; Peterson EC. Oxygen diffusion into mammalian cells following ultrahigh dose rate irradiation and lifetime estimates of oxygen sensitive species. Radiat. Res. 1978 76, 522-532.

274. Ling CC ; Michaels HB ; Gerweck LE ; Epp ER ; Peterson EC. Oxygen sensitization of mammalian cells under different irradiation conditions. Radiat. Res. 1981 86, 325-340.
275. Liquier J ; Fort D ; Nguyen D ; Cao A ; Taillandier E. DNA protection by aminothiols: Study of the cysteamine-DNA interaction by vibrational spectroscopy. Int. J. Biol. Macromol. 1983 5, 89-93.
276. Littbrand B. Survival characteristics of mammalian cell lines after single or multiple exposures to roentgen radiation under oxic or anoxic conditions. Acta Radiol. Ther. Phys. Biol. 1970 9, 257-281.
277. Littbrand B ; Révész L. The effect of oxygen on cellular survival and recovery after radiation. Br. J. Radiol. 1969 42, 914-924.
278. Livesey JC ; Reed DJ. Measurement of glutathione-protein mixed disulfides. Int. J. Radiat. Oncol. Biol. Phys. 1984 10 Suppl., In press.
279. Lote K. Hypoxic radioprotection by temporary intestinal ischemia: Degradable starch microsphere embolization in the cat. Am. J. Roentgenol. 1981 137, 909-914.
280. Lötscher HR ; Winterhalter KH ; Carafoli E ; Richter, C. Hydroperoxides can modulate the redox state of pyridine nucleotides and the calcium balance in rat liver mitochondria. Proc. Natl. Acad. Sci. U.S.A. 1979 76, 4340-4344.
281. Lunec J. Introductory review. Involvement of ADP-ribosylation in cellular recovery from some forms of DNA damage. In: "Cellular Repair of Radiation Damage: Mechanisms and Modifying Agents," (T Alper ; WA Cramp ; S Hornsey ; JS Orr ; TE Wheldon, eds.) 1984, Br. J. Cancer, v.49, Suppl.6, 317pp.
282. Lvovsky EA. Interferon - its role in radioprotection as a hematopoietic stem cell stimulator. Int. J. Radiat. Oncol. Biol. Phys. 1981 7, 1290-1291.
283. Lynch RE ; Fridovich I. Permeation of erythrocyte stroma by superoxide radical. J. Biol. Chem. 1978 253, 4697-4699.
284. Magee JL ; Chatterjee A. A spur unfolding model for the radiolysis of water. Radiat. Phys. Chem. 1980 15, 125-132.
285. Mahler RH ; Mehrotra BD. Dependence of dioxyribonucleic-amine interactions on deoxyribonucleic acid compositions. Biochim. Biophys. Acta 1962 55, 252.
286. Mahler RH ; Mehrotra BD. The interaction of nucleic acids with diamines. Biochim. Biophys. Acta 1963 68, 211.
287. Mallet G ; Costa A ; Rix-Montel MA ; Vasilescu D. Influence of ionizing radiations on DNA in presence of sulfur containing radioprotectors. 1. Weak dose effect of γ-radiations on DNA solutions in absence of radioprotectors. Stud. Biophys. 1982 91, 167-176.
288. Mannervik B. Thioltransferases. In: "Enzymatic Basis of Detoxication," (WB Jakoby, ed.) 1980, v.2, Chapt.12, pp.229-244. Academic Press, New York.
289. Manney TR ; Brustad TB ; Tobias CA. Effects of glycerol and of anoxia on the radiosensitivity of haploid yeasts to densely ionizing particles. Radiat. Res. 1963 18, 374-388.
290. Marcovich H. Sur le mécanisme de l'activité radioprotectrice de la cystéamine chez les bactéries. Annales Inst. Pasteur, Paris 1957 93, 456-462.
291. Matheson MS ; Rabani J. Pulse radiolysis of aqueous hydrogen solutions. I. Rate constants for reaction of e_{aq}^- with itself and other transients. II. The interconvertibility of e_{aq}^- and H^1. J. Phys. Chem. 1965 69, 1324-1335.

292. McCay PB ; King MM. Vitamin E: Its role as a biologic free radical scavenger and its relationship to the microsomal mixed-function oxidase system. In: "Vitamin E, a Comprehensive Treatise," (LJ Machlin, ed.) 1980, 289-317. Marcel Dekker, Inc. New York.
293. McComb DJ ; Kovacs K ; Horner HC ; Gallagher GT ; Schwedes U ; Usadel KH ; Szabo S. Cysteamine-induced adrenocortical necrosis in rats. Exp. Molec. Pathol. 1981 35, 422-434.
294. McDonald MR. The inactivation of dilute solutions of crystalline trypsin by x-radiation. Radiat. Res. 1955 3, 337.
295. McIntyre TM ; Curthoys NP. The interorgan metabolism of glutathione. Int. J. Biochem. 1980 12, 545-551.
296. Meister A ; Anderson ME. Glutathione. Ann. Rev. Biochem. 1983 52, 711-760.
297. Mendiondo OA ; Grigsby PW ; Beach JL. Radioprotection combined with hypoxic sensitization during radiotherapy of a solid murine tumor. Radiology 1983 148, 291-293.
298. Meredith MJ ; Reed DJ. Depletion in vitro of mitochondrial glutathione in rat hepatocytes and enhancement of lipid peroxidation by adriamycin and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Biochem. Pharmacol. 1983 32, 1383-1388.
299. Meredith MJ ; Reed DJ. Status of the mitochondrial pool of glutathione in the isolated hepatocyte. J. Biol. Chem. 1982 257, 3747-3753.
300. Mesnard D ; Miginiac L ; Fatome M ; Laval JD ; Sentenac-Roumanou H ; Lion C. Synthesis and radiopharmacological study of unsaturated derivatives of cysteamine. Eur. J. Med. Chem. Chim. Ther. 1980 15, 247-252.
301. Michael BD ; Adams GE ; Hewitt HB ; Jones MBG ; Watts ME. A post-effect of oxygen in irradiated bacteria: A submillisecond fast mixing study. Radiat. Res. 1973 54, 239-251.
302. Michael BD ; Harrop HA. Time scale and mechanism of radiosensitization and radioprotection at the cellular level. In: "Radiatiation Sensitizers: Their Use in Clin. Manage. Cancer," (Proc. Conf. Meeting, 1979, New York) (LW Brady, ed.) 1980, pp.14-21. Masson Publ., New York.
303. Michael BD ; Harrop HA ; Held KD. Time scale and mechanisms of the oxygen effect in irradiated bacteria. In: "Oxygen and Oxy-Radicals in Chemistry and Biology," (MAJ Rodgers ; EL Powers, eds.) 1981, pp.285-292. Academic Press, New York.
304. Michaelson SM. Biological effects and health hazards of radiofrequency and microwave energy; fundamentals and overall phenomenology. In: "Biological Effects and Dosimetry of Nonionizing Radiation - Radiofrequency and Microwave Energies," (M Grandolfo ; SM Michaelson ; A Rindi, eds.) 1983, pp.337-357. NATO Scientific Affairs Division. Plenum Press, New York.
305. Michaelson SM ; Schwan HP. Factors governing the use of microwave/radiofrequency energies in cancer therapy. Adv. Radiat. Biol. 1981 9, 323-409.
306. Milas L ; Hunter N ; Ito H ; Peters LJ. Effect of tumor type, size, and endpoint on tumor radioprotection by WR-2721. Int. J. Radiat. Oncol. Biol. Phys. 1984 10, 41-48.
307. Milas L ; Hunter N ; Reid BO. Protective effects of WR-2721 against radiation-induced injury of murine gut, testis, lung, and lung tumor nodules. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 535-538.

308. Milas L ; Hunter N ; Reid BO ; Thames HD Jr. Protective effects of S-2-(3-aminopropylamino)ethylphosphorothioate against radiation damage of normal tissues and a fibrosarcoma in mice. Cancer Res. 1982 42, 1888-1897.
309. Millar BC ; Fielden EM ; Steele JJ. A biphasic radiation survival response of mammalian cells to molecular oxygen. Int. J. Radiat. Biol. 1979 36, 177-180.
310. Mishra VS ; Srivastava PN. Radioprotective effect of MPG and WR-2721 against γ-radiolysis of human placental alkaline phosphatase. Proc. Natl. Acad. Sci., India. Sect. B 1981 51, 318-324.
311. Misustova J ; Novak L. The importance of hypothermy in the mechanism of the protective action of sodium fluoroacetate. In: "Radioprotection and Sensitization," (HL Moroson ; M Quintiliani, eds.) 1970, pp.343-347. Taylor and Francis, London.
312. Mitchel JB ; Russo A. Thiols, thiol depletion and thermosensitivity. Radiat Res. 1983 95, 471-485.
313. Mitchel RE ; Morrison DP. Assessment of the role of oxygen and mitochondria in heat shock induction of radiation and thermal resistance in Saccharomyces cerevisiae. Radiat. Res. 1983 96, 113-117.
314. Mitchel RE ; Morrison DP ; Unrau P. Assessment of the oxygen effect, and oxygen modification of OH· damage, on radiation-induced lethality and gene conversion in Saccharomyces cerevisiae. Radiat. Res. 1982 89, 528-536.
315. Mitchell JB ; Russo A ; Kinsella TJ ; Glatstein E. Glutathione elevation during thermotolerance induction and thermosensitization by glutathione depletion. Cancer Res. 1983 43, 987-991.
316. Modig HG ; Edgren M ; Revesz L. Effect of radioprotective aminothiols on the induction and repair of single-strand breaks in the DNA of irradiated mammalian cells. Acta Radiol. Ther. Phys. Biol. 1977 16, 245-256.
317. Morehouse LA ; Tien M ; Bucher JR ; Aust SD. Effect of hydrogen peroxide on the initiation of microsomal lipid peroxidation. Biochem. Pharm. 1983 32, 123-127.
318. Mottram JC. Factors of importance in radiosensitivity of tumours. Br. J. Radiol. 1936 9, 606-614.
319. Nakatsugawa S ; Sugahara T. Effects of inhibitors of radiation-induced potentially lethal damage repair on chemotherapy in murine tumors. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 1555-1559.
320. Nanni EJ Jr ; Angelis CT ; Dickson J ; Sawyer DT. Oxygen activation by radical coupling between superoxide ion and reduced methyl viologen. J. Am. Chem. Soc. 1981 103, 4268-4270.
321. National Research Council, Committee on the Biological Effects of Ionizing Radiations. "The Effects on Populations of Exposure to Low Levels of Ionizing Radiation," 1980, Natl. Acad. Sci., Wash., D.C.
322. Nowotny A ; Behling UH ; Chang H. Relationship of structure to function in bacterial endotoxins. VIII. Biological activities in a polysaccharide-rich fraction. J. Immunol. 1975 116, 199-203.
323. Oberly LW (ed.). "Superoxide Dismutase," 1982, v.1, 152pp. and v.2, 177pp. CRC Press, Boca Raton, FL.
324. Ohno T ; Nishimura T ; Nakano K ; Kaneko I. Differential recovery from potentially lethal damage in normal human lung fibroblasts after irradiation with Co-60 γ-rays and accelerated N-ion beam. Int. J. Radiat. Biol. 1984 46, 21-26.

325. Olontseva OI ; Drozhennikov VA ; Lyashenko VA ; Perevezentseva OS ; Orlova EB ; Kalistratov GV. Biological activity of purified spleen extracts containing an inhibitor of DNase I, under the effect of radiation. Radiobiologiya 1983 23, 259-263.
326. Ormerod MG ; Alexander P. On the mechanism of radiation protection by cysteamine: An investigation by means of electron spin resonance. Radiat. Res. 1963 18, 495-509.
327. Ormstad K ; Jones DP ; Orrenius S. Characteristics of glutathione biosynthesis by freshly isolated rat kidney cells. J. Biol. Chem. 1980 255, 175-181.
328. Ormstad K ; Lastbom T ; Orrenius S. Characteristics of renal glutathione oxidase activity. F.E.B.S. Lett. 1981 130, 239-243.
329. Ormstad K ; Lastbom T ; Orrenius S. Evidence for different localization of glutathione oxidase and γ-glutamyltransferase activities during extracellular glutathione metabolism in the isolated perfused kidney. Biochim. Biophys. Acta 1982 700, 148-153.
330. Ormstad K ; Lastbom T ; Orrenius S. Translocation of amino acids and glutathione studied with the perfused kidney and isolated renal cells. F.E.B.S. Lett. 1980 112, 55-59.
331. Ormstad K ; Moldeus P ; Orrenius S. Partial characterization of a glutathione oxidase present in rat kidney plasma membrane fraction. Biochem. Biophys. Res. Commun. 1979 89, 497-503.
332. Ormstad K ; Orrenius S ; Lastbom T ; Uehara N ; Pohl J ; Stehar J ; Brock N. Pharmacokinetics and metabolism of sodium-2-mercaptoethane sulfonate (Mesna) in the rat. Cancer Res. 1983 43, 333-338.
333. Orr JS. Concepts, problems and the role of modifying agents in the relationship between recovery of cells' survival ability and the mechanisms of repair of radiation lesions. In: "Cellular Repair of Radiation Damage: Mechanisms and Modifying Agents," (T Alper ; WA Cramp ; S Hornsey ; JS Orr ; TE Wheldon, eds.) 1984, Br. J. Cancer, v.49, Suppl.6, pp. 1-6.
334. Orrenius S ; Ormstad K ; Thor H Jewell S. Turnover and functions of glutathione studied with isolated hepatic and renal cells. Fed. Proc. 1983 42, 3177-3188.
335. Ortaldo JR ; McCoy JL. Protective effects of interferon in mice previously exposed to lethal irradiation. Radiat. Res. 1980 81, 262-266.
336. Owen TC ; Wilbraham AC. Glutathione-sulfonic acid, the predominant product of x-radiolysis of air saturated solutions of oxidized glutathione. Radiat. Res. 1972 50, 253-260.
337. Owen TC ; Wilbraham AC. The radiation chemistry of biochemical disulfides. II. Lipoic acid. J. Am. Chem. Soc. 1969 91, 3365-3371.
338. Owen TC ; Wilbraham AC ; Noach JAG ; Ellis DR. Reduced products and hydrogen from x-irradiated air-saturated solutions of cystamine and cystine. Radiat. Res. 1972 50, 234-252.
339. Palladino MA ; Galton JE ; Troll W ; Thorbecke GJ. γ-Irradiation-induced mortality: Protective effect of protease inhibitors in chickens and mice. Int. J. Radiat. Biol. 1982 41, 183-191.
340. Parrish JA ; Anderson RR ; Urbach F ; Pitts D (eds.). "Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Long Wave Ultraviolet," 1978, 262pp. John Wiley and Sons, New York.

341. Paterson MC ; Bech-Hansen NT ; Smith PJ ; Mulvihill JJ. Radiogenic neoplasia, cellular radiosensitivity, and faulty DNA repair. In: "Radiation Carcinogenesis: Epidemiology and Biological Significance, Progress in Cancer Research," (JD Jr Boice ; JF Jr Fraumeni, eds.) 1983, v.26, pp.319-336. Raven Press, New York.
342. Patt HM. Protective mechanisms in ionizing radiation injury. Physiol. Rev. 1953 33, 35-76.
343. Patt HM ; Tyree EB ; Straube RL ; Smith DE. Cysteine protection against X-irradiation. Science 1949 110, 213-214.
344. Peak MJ ; Peak JG. Protection by glycerol against the biological actions of near UV light. Radiat. Res. 1980 83, 553-558.
345. Petkau A. Radiation protection by superoxide dismutase. Photochem. Photobiol. 1978 28, 765-774.
346. Phillips RA ; Tolmach LJ. Repair of potentially lethal damage in X-irradiated HeLa cells. Radiat. Res. 1966 29, 413-432.
347. Pihl A ; Eldjarn L. The formation and biological role of mixed disulphides. In: "Fourth Int. Congr. Biochem., 1958, Vienna." 1959, v.13, pp.43-62. Pergamon Press, London.
348. Pihl A ; Eldjarn L ; Bremner J. On the mode of action of X-ray protective agents. III. The enzymatic reduction of disulfides. J. Biol. Chem. 1957 227, 339-345.
349. Pihl A ; Sanner T. Chemical protection against ionizing radiation by sulphur-containing agents. In: "Radioprotection and Sensitization," (HL Moroson ; M Quintiliani, eds.) 1970, pp.43-55. Taylor and Francis, London.
350. Pihl A ; Sanner T. Protection of sulfhydryl compounds against ionizing radiation. Biochim. Biophys. Acta 1963 78, 537-539.
351. Pizzarello DJ (ed.). "Radiation Biology," 1982, 298pp. CRC Press, Inc., Boca Raton, Florida.
352. Pizzarello DJ ; Witcofski RL. "Medical Radiation Biology," 1982, 164pp. Lea and Febiger, Philadelphia.
353. Port, CD ; Ward WF. The ultrastructure of radiation injury in rat lung: Modification by D-penicillamine. Radiat. Res. 1982 92, 61-82.
354. Pospisil M ; Jary J ; Netikova J ; Marek M. Glucan-induced enhancement of hemopoietic recovery in γ-irradiated mice. Experientia 1982 38, 1232-1234.
355. Potop I ; Briese M-A ; Savulescu M. The influence of thymosterin B on the number of leukocytes in the peripheral blood of rats irradiated with X-rays. Rev. Roum. Med. Endocrinol. 1982 20, 177-180.
356. Powers EL. O_2 and cellular radiation sensitivity. In: "Oxygen and Oxy-Radicals in Chemistry and Biology," (MAJ Rodgers ; EL Powers, eds.) 1981, pp.241-246. Academic Press, New York.
357. Powers EL. Responses of cells to radiation sensitizers: Methods of analysis. Int. J. Radiat. Res. 1982 42, 629-651.
358. Powers EL ; Webb RB ; Ehret CF. Storage transfer and utilization of energy from X-rays in dry bacterial spores. Radiat. Res. 1960 Suppl. 2, 94-121.
359. Powers EL ; Webb RB ; Kaleta BF. Oxygen and nitric oxide as modifiers of radiation injury in spores of Bacillus megaterium. Proc. Natl. Acad. Sci. U.S.A. 1980 46, 984-993.
360. Powis G ; Svingen BA ; Appel P. Quinone-stimulated superoxide formation by subcellular fractions, isolated hepatocytes, and other cells. Molec. Pharmacol. 1981 20, 387-394.

361. Prasad KN. Acute radiation syndromes. In: "Radiation Biology," (DJ Pizzarello, ed.) 1982, pp.205-235. CRC Press Inc., Boca Raton, Florida.
362. Pryor WA. Free radical reactions and their importance in biochemical systems. Fed. Proc. 1973 32, 1862-1868.
363. Purdie JW. γ-Radiolysis of cystine in aqueous solution. Dose rate effects and a proposed mechanism. J. Am. Chem. Soc. 1967 89, 226-230.
364. Purdie JW. γ-Radiolysis of disulfides in aqueous solution. II. D-penicillamine disulfide. Can. J. Chem. 1969 47, 1029-1036.
365. Purdie JW. γ-Radiolysis of disulfides in aqueous solution. III. L-cystine-D-penicillamine mixed disulfide. Can. J. Chem. 1969 47, 1037-1043.
366. Purdie JW ; Inhaber ER ; Schneider H ; Labelle JL. Interaction of cultured mammalian cells with WR-2721 and its thiol, WR-1065: Implications for mechanisms of radioprotection. Int. J. Radiat. Biol. 1983 43, 517-527.
367. Quintiliani M ; Badiello R ; Tamba M. Radiolysis of glutathione in oxygen-containing solutions of pH 7. Int. J. Radiat. Biol. 1977 32, 195-202.
368. Quintiliani M ; Boccacci M. Factors affecting the in vitro inactivation of aldolase by X-rays. Int. J. Radiat. Biol. 1963 7, 255-264.
369. Raleigh JA ; Kremers W ; Gaboury B. Dose-rate and oxygen effects in models of lipid membranes: Linoleic acid. Int. J. Radiat. Biol. 1977 31, 203-213.
370. Raleigh JA ; Shum FY. Radiation peroxidation in micellar fatty acids. (Third Int. Conf. on Oxygen Radicals, July 1983, Munich) 1983, In press.
371. Raleigh JA ; Shum FY. Radioprotection in model lipid membranes by hydroxyl radical scavengers: Supplementary role for α-tocopherol in scavenging secondary peroxy radicals. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.87-102. Academic Press, New York.
372. Reed DJ. Cellular defense mechanisms against reactive metabolites. In: "Biochemistry of Foreign Compounds," (MW Anders, ed.) 1984. In press. Academic Press, New York.
373. Reed DJ ; Beatty P. The role of the cystathionine pathway in glutathione regulation by isolated hepatocytes. In: "Functions of Glutathione in Liver and Kidney," (H Sies ; A Wendel, eds.) 1978, pp.13-21. Springer-Verlag, Berlin.
374. Reed DJ ; Fariss MW. Glutathione depletion and susceptibility. Pharmacol. Rev. 1984 36, 25S-33S.
375. Reed DJ ; Meredith MJ. Glutathione conjugation systems and drug disposition. In: "Drugs and Nutrients," (DA Roe ; TC Campbell, eds.) 1984, Chapt. 6, pp.179-224. Marcel Dekker, Inc., New York.
376. Reed DJ ; Orrenius S. The role of methionine in glutathione biosynthesis by isolated hepatocytes. Biochem. Biophys. Res. Commun. 1977 77, 1257-1264.
377. Révész L. Studies with glutathione-deficient human cells. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.237-253. Academic Press, New York.
378. Révész L ; Edgren M. Glutathione-dependent yield and repair of single-strand DNA breaks in irradiated cells. In: "Cellular Repair of Radiation Damage: Mechanisms and Modifying Agents," (T Alper ; MA Cramp ; S Hornsey ; JS Orr ; TE Wheldon, eds.) 1984, Br. J. Cancer, v.49, Suppl.6, pp. 55-60.

379. Révész L ; Edgren M. Mechanisms of radiosensitization and protection studied with glutathione-deficient human cell lines. In: "Prog. Radio. Oncol., Pap. Int. Meet. 2nd," (K-H Kaercher ; HD Kogelnik ; G Reinartz, eds.) 1982, pp.235-242. Raven Press, New York.
380. Révész L ; Edgren M ; Nishidai T. Mechanisms of inherent radioprotection in mammalian cells. In: "Modification of Radiosensitivity in Cancer Treatment," (T Sugahara, ed.) 1984, Chapt.2, pp.13-29. Academic Press, New York.
381. Révész L ; Littbrand B. Variation of the relative sensitivity of closely related neoplastic cell lines irradiated in culture in the presence or absence of oxygen. Nature 1964 203, 742-744.
382. Révész L ; Littbrand B ; Hidander J ; Scott OCA. Oxygen effects in the shoulder region of cell survival curves. In: "Proc. 6th C.H. Gray Conf., 1974," (T Alper, ed.) 1975, pp.141-149. John Wiley & Sons, New York.
383. Révész L ; Malaise EP. Significance of cellular glutathione in radioprotection and repair of radiation damage. In: "Functions of Glutathione," (A Larsson, ed.) 1983, pp.161-170. Raven Press, New York.
384. Riklis E. DNA repair as a probe of radiosensitivity and radioprotection. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.363-379. Academic Press, New York.
385. Riklis E ; Hagen MP ; Catravas GN. Modification of cell survival and DNA repair capacity by WR-2721 following irradiation. Radiat. Res. 1982 91, 374-375.
386. Ritter MA ; Brown DQ ; Glover DJ ; Yuhas JM. *In vitro* studies on the absorption of WR-2721 by tumors and normal tissues. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 523-526.
387. Rix-Montel MA ; Mallet G ; Costa A ; Vasilescu D. Influence of ionizing radiations on DNA in the presence of sulfur containing radioprotectors. 2. Cysteamine protection against γ-radiations. Stud. Biophys. 1982 89, 205-212.
388. Robison SH ; Cantoni O ; Heck JD ; Costa M. Soluble and unsoluble nickel compounds induce DNA repair synthesis in cultured mammalian cells. Cancer Lett. 1983 17, 273-279.
389. Romantsev EF. Effect of L-cysteine and 2-mercaptoethylamine on the venous oxygen content of rats. Med. Radiol. 1960 5, 19-21.
390. Roots R ; Okada S. Protection of DNA molecules of cultured mammalian cells from radiation-induced single-strand scissions by various alcohols and SH compounds. Int. J. Radiat. Biol. 1972 21, 329-342.
391. Rothe WE ; Grenen MM ; Wilson SM. Radioprotection of mice by hypoxia and chemical agents. Nature 1963 198, 403.
392. Roy RM ; Malick MA ; Clark GM. Increased hematopoietic stem cell survival in mice injected with tocopherol after X-irradiation. Strahlentherapie 1982 158, 312-318.
393. Rumyantseva GV ; Weiner LM ; Molin YN ; Budker VG. Permeation of liposome membrane by superoxide radical. F.E.B.S. Lett. 1979 108, 477-480.
394. Saez G ; Thornalley PJ ; Hill HAO ; Hems R ; Bannister JV. The production of free radicals during the autoxidation of cysteine and their effect on isolated rat hepatocytes. Biochim. Biophys. Acta 1982 719, 24-31.
395. Sandu VD ; Abraham AD ; Uray Z. Effects of treatments with methylandrostadienolone associated with thymic extracts on the small intestine of irradiated rats. Stud. Cercet. Biol. (Clui) 1982 34, 50-52.

396. Sanner T ; Pihl A. Significance and mechanism of the indirect effect in bacterial cells. The relative protective effect of added compounds in Escherichia coli B, irradiated in liquid and in frozen suspension. Radiat. Res. 1969 37, 216-227.
397. Sato PH. Commentary: Pharmacologic prospects of the therapeutic use of administered enzymes. Drug Metab. Disposition 1984 12, 1-3.
398. Sato T ; Nakamura W. Radioprotective effect of 5-hydroxytryptophan and 5-hydroxytryptamine in mastocytoma cells against irradiation in vitro. Nippon Igaku Hoshasen Gakkai Zasshi 1981 41, 868-872.
399. Sawyer DT ; Valentine JS. How super is superoxide? Acc. Chem. Res. 1981 14, 393-400.
400. Schafer K ; Bonifacic M ; Bahnemann D ; Asmus K-D. Addition of oxygen to organic sulfur radicals. J. Phys. Chem. 1978 82, 2777-2780.
401. Schanne FAX ; Kane AB ; Young EE ; Farber JL. Calcium dependence of toxic cell death: A final common pathway. Science 1979 206, 700-702.
402. Schuman VL ; Levitt SH ; Song CW. The radioprotective effect of 5-thio-D-glucose on normal tissues in vivo. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 589-591.
403. Schuurhuis GJ ; Hommes J ; Vos J ; Molenaar I ; Konings AWT. Radiation induced structural changes in membrane proteins of human erythrocytes and ghosts and their relation to cellular morphology. Int. J. Radiat. Biol. 1984 45, 159-177.
404. Schwartz D. The effect of oxygen concentration on X-ray-induced chromosome breakage in maize. Proc. Natl. Acad. Sci. U.S.A. 1952 38, 490-494.
405. Schwarz HA. Applications of the spur diffusion model to the radiation chemistry of aqueous solutions. J. Phys. Chem. 1969 73, 1928-1937.
406. Scott OCA. Chemical protection in mammals. In: "Radiation Effects in Physics, Chemistry and Biology," (M Ebert ; A Howard, eds.) 1963, pp.294-304. North Holland Publ. Co., Amsterdam.
407. Sheldon PW ; Chu AM. The effect of anesthetics on the radiosensitivity of a murine tumor. Radiat. Res. 1979 79, 568-578.
408. Shenoy MA ; Asquith JC ; Adams GE ; Michael BD ; Watts ME. Time-resolved oxygen effects in irradiated bacteria and mammalian cells: A rapid-mix study. Radiat. Res. 1975 62, 498-512.
409. Sies H. (ed.). "Oxidative Stress," 1984, In press. Academic Press, New York.
410. Sies H ; Wefers H ; Graf P ; Akerboom TPM. Hepatic hydroperoxide metabolism: Studies on redox cycling and generation of H_2O_2. In: "Isolation, Characterization, and Use of Hepatocytes," (RA Harris ; NW Cornell, eds.) 1983, pp.341-348. Elsevier. New York.
411. Sies H ; Wendel A ; Bors W. Metabolism of organic hydroperoxides. In: "Metabolic Basis of Detoxication," 1982, Chapt. 16, pp.307-321. Academic Press, New York.
412. Sigdestad CP. Assessment of antiradiation drug effectiveness to fission neutron irradiation. 1981. Available through NTIS AD-A108 295/7.
413. Silbernagl S ; Pfaller W ; Heinle H ; Wendel A. Topology and function of renal γ-glutamyltranspeptidase. In: "Functions of Glutathione in Liver and Kidney," (H Sies ; A Wendel, eds.) 1978, pp.60-69. Springer-Verlag, Berlin.
414. Simonyan MA ; Minasyan GM. Effect of superoxide dismutase on the viability of rats irradiated by X-rays. Zh. Eksp. Klin. Med. 1981 21, 37-39.

415. Singh A ; Singh H. Time-scale and nature of radiation-biological damage: Approaches to radiation protection and post-irradiation therapy. Prog. Biophys. Mol. Biol. 1982 39, 69-107.
416. Sjöberg L ; Eriksen TE ; Revesz L. The reaction of the hydroxyl radical with glutathione in neutral and alkaline aqueous solution. Radiat. Res. 1982 89, 255-263.
417. Smith DW ; Schaller HE ; Bonhueffer FJ. DNA synthesis in vitro. Nature 1970 226. 711-713.
418. Smith MT ; Thor H ; Orrenius S. Toxic injury to isolated hepatocytes is not dependent on extracellular calcium. Science 1981 213, 1257-1259.
419. Sobolev AS ; Chirkov YY. β-Adrenergic mechanism of mammalian cells. Radioprotection by isoproterenol. Strahlentherapie 1982 158, 747-751.
420. Soboll S ; Scholz R ; Freisl M ; Elbers R ; Heldt HW. Distribution of metabolites between mitochondria and cytosol of perfused liver. In: "Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies," (JM Tager ; HD Söling ; JR Williamson, eds.) 1976, pp.29-40. North-Holland Publ. Co., Amsterdam.
421. Sodicoff M ; Conger AD. Radioprotection of the rat parotid gland by WR-2721 and isoproterenol and its modification by propranolol. Radiat. Res. 1983 94, 97-104.
422. Sodicoff M ; Conger AD ; Pratt ME ; Sinesi M ; Trepper P. Chemoradioprotection of the rat parotid gland by combined use of WR-2721 and Ro-07-0582. Radiat. Res. 1979 80, 348-353.
423. Sokoloff B. Carcinoid and Serotonin. Chapt. VI, Serotonin in Radiation Injury, Recent Results in Cancer Res. 1968 15, 83-95.
424. Staib AH ; Effler K. Strahlenschutzwirkung von cholinomimetika. Die Naturwissenschaften 1966 53, 583.
425. Stein G ; Swallow AJ. The biological action of ionising radiations from the point of view of radiation chemistry. In: "Advances in Radiobiology," (GC DeHevesy ; AR Gunner ; JD Abbatt, eds.) 1956, pp.16-21. Oliver & Boyd, Edinburgh.
426. Stevenson AFG ; Monig H ; Weskesser J. Radioprotective and hemopoietic effects of some lipopolysaccharides from Rhodospirillaceae species in mice. Experientia 1981 37, 1331-1332.
427. Stewart FA ; Rojas A. Radioprotection of mouse skin by WR-2721 in single and fractionated treatments. Br. J. Radiol. 1982 55, 42-47.
428. Stratford IJ ; Maughan RL ; Michael BD. The decay of potentially lethal oxygen-dependent damage in fully hydrated Bacillus megaterium spores exposed to pulsed electron irradiation. Int. J. Radiat. Biol. 1977 32, 447-455.
429. Sümegi J ; Sanner T ; Pihl A. Inactivation of DNA-dependent RNA polymerase from Escherichia coli by X-rays in solution. Biochem. Biophys. Acta 1972 262, 145-153.
430. Sümegi J ; Sanner T ; Pihl A. Protection of RNA polymerase against ionizing radiation by mixed disulphide formation. Int. J. Radiat. Biol. 1971 20, 397-399.
431. Sweeney TR. A survey of compounds from the antiradiation drug development program of the U.S. Army Medical Research and Development Command. 1979, Walter Reed Army Inst. Res. Wash.D.C.
432. Sweet JP ; Thomas JK. Absolute rate constants for H atom reactions in aqueous solutions. J. Phys. Chem. 1964 68, 1363-1368.
433. Sykes P. "A Guideline to Mechanisms in Organic Chemistry," 1975, 362pp. John Wiley & Sons, New York.

434. Takeda A ; Katoh N ; Yonezawa M. Restoration of radiation injury by ginseng. 3. Radioprotective effect of thermostable fraction of ginseng extract on mice rats and guinea-pigs. J. Radiat. Res. 1982 23, 150-167.
435. Takeda A ; Yonezawa M ; Katoh N. Restoration of radiation injury by ginseng panax-ginseng. 1. Responses of X-irradiated mice to ginseng extract. J. Radiat. Res. 1981 22, 323-335.
436. Tallentire A ; Jones AB ; Jancobs GP. The radiosensitizing actions of ketonic agents and oxygen in bacterial spores suspended in aqueous and non-aqueous milleux. Isr. J. Chem. 1972 10, 1185-1197.
437. Tamba M ; Quintiliani M. Kinetic studies of reactions involved in hydrogen transfer from glutathione to carbohydrate radicals. Radiat. Phys. Chem. 1984 23, 259-263.
438. Tanaka Y ; Akagi K ; Sokawa K ; Sugahara T. PLD repair inhibitors as radiosensitizer and clinical trials. Int. J. Radiat. Oncol. Biol. Phys. 1984 10(Suppl.) In press.
439. Tappel AL. Measure of and protection from in vivo lipid peroxidation. In: "Biochemical and Clinical Aspects of Oxygen," (WA Caughey, ed.) 1979, pp.679-698. Academic Press, New York.
440. Thomas JK. Rates of reaction of the hydroxyl radical. Trans. Faraday Soc. 1965 61, 702-707.
441. Thomas JK ; Rabani J ; Matheson MS ; Hart EJ ; Gordon S. Absorption spectrum of the hydroxyl radical. J. Phys. Chem. 1966 70, 2409-2410.
442. Tien M ; Svingen BA ; Aust SD. Superoxide dependent lipid peroxidation. Fed. Proc. 1981 40, 179-182.
443. Travis EL ; De Luca AM ; Fowler JF ; Padikal TN. The time course of radioprotection by WR 2721 in mouse skin. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 843-850.
444. Travis EL ; Parkins CS ; Holmes SJ ; Down JD ; Fowler JF. WR-2721 protection of pneumonitis and fibrosis in mouse lung after single doses of X-rays. Int. J. Radiat. Oncol. Biol. Phys. 1984 10, 243-251.
445. Tremblay GY ; Daniels MJ ; Schaechter M. Isolation of a cell membrane - DNA-nascent RNA complex from bacteria. J. Molec. Biol. 1969 40, 65-76.
446. Trocha PJ ; Catravas GN. Effects of WR 2721 on cyclic nucleotide levels and lysosomal enzyme activities. 1981. Available through NTIS AD-A121 250/5.
447. Trosko JE ; Chang CC. The role of radiation and chemicals in the induction of mutations and epigenetic changes during carcinogenesis. Adv. Radiat. Biol. 1981 9, 1-36.
448. Tsao B ; Curthoys NP. The absolute asymmetry of orientation of γ-glutamyltranspeptidase and aminopeptidase on the external surface of the rat renal brush border membrane. J. Biol. Chem. 1980 255, 7708-7711.
449. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and effects of ionizing radiation. In: "Report to the General Assembly, with Annexes," 1977, 725pp. United Nations, New York.
450. Uray Z ; Ban C ; Maniu M ; Bucur M ; Carpen M. Usefullness of leukotropin as an adjuvant in anti-tumor radiotherapy. Agressologie 1980 21, 215-218.
451. Urbach F ; Forbes PD ; Davies RE. Modification of photocarcinogenesis by chemical agents. J. Natl. Cancer Inst. 1982 68, 229-235.
452. Usdin E ; Eckert H ; Forrest IS. (eds.). "Phenothazines and Structurally Related Drugs: Basic and Clinical Studies," 1980, 376pp. Elsevier, New York.

453. Utley, JF ; Quinn CA ; White FC ; Seaver NA ; Bloor CM. Protection of normal tissue against late radiation injury by WR-2721. Radiat. Res. 1981 86, 408-415.
454. Van Bekkum DW ; De Groot J. Observations on chemical protection in vivo and in vitro. In: "Progress in Radiobiology," (JS Mitchell ; BE Holmes ; CL Smith, eds.) 1956, pp.243-248, Oliver & Boyd, Edinburgh.
455. Van den Brenk HAS ; Elliott K. Radioprotective action of 5-hydroxytryptamine. Nature 1958 182, 1506-1507.
456. Van den Brenk HAS ; Haas M. Studies of mechanisms of chemical radiation protection in vivo. I. 5-hydroxytryptamine in relation to effect of antimetabolites antogonists and releasing agents. Int. J. Radiat. Biol. 1961 3, 73-94.
457. Van den Brenk HAS ; Jamieson D. Studies of mechanisms of chemical radiation protection in vivo. II. Effect of high pressure oxygen on radioprotection in vivo and its relationship to 'oxygen poisoning'. Int. J. Radiat. Biol. 1962 4, 379-402.
458. Van der Meer C ; Valkenburg PW. The pharmacology of KCN as a prophylactic against radiation. Biochem. Pharmacol. 1961 7, 237-247.
459. Van der Meer C ; Valkenburg PW ; Remmelts R. Effect of radioprotective sulphydryl compounds on the oxygen tension in the spleen of mice. Nature 1961 189, 588-589.
460. Van der Meer C ; Van Bekkum DW. A study on the mechanism of radiation protection by 5-hydroxytryptamine and tryptamine. Int. J. Radiat. Biol. 1961 4, 105-110.
461. Van der Meer C ; Van Bekkum DW. The mechanism of radiation protection by histamine and other biological amines. Int. J. Radiat. Biol. 1959 1, 5-23.
462. Vasilescu D ; Rix-Montel MA. Interaction of sulfur-containing radioprotectors with DNA: A spectrophotometric study. Physiol. Chem. Phys. 1980 12, 51-55.
463. Vergroesen AJ ; Budke L ; Vos O. Protection against x-irradiation by sulphydryl compounds. II. Studies on the relation between chemical structure and protective activity for tissue culture cells. Int. J. Radiat. Biol. 1967 13, 77-92.
464. Verly WG ; Grégoire S ; Rayet P ; Urbain MF. Metabolism of β-mercaptoethylamine 1. in mice. Biochem. J. 1954 58, 660-662.
465. Verly WG ; Koch G. Metabolism of β-mercaptoethylamine 2. in the dog. Biochem. J. 1954 58, 663.
466. Vignais PM ; Vignis PV. Fuscin, an inhibitor of mitochondrial SH-dependent transport-linked functions. Biochim. Biophys. Acta 1973 325, 357-374.
467. Vigneulle RM ; Baum SJ. Effects of endotoxin on survival of hypertransfused mice. Exp. Hematol. 1982 10(Suppl.12), 249-262.
468. Vina J ; Hems R ; Krebs HA. Maintenance of glutathione content in isolated hepatocytes. Biochem. J. 1978 170, 627-630.
469. Vina J ; Romero FJ ; Saez GT ; Pallardo FV. Effects of cysteine and N-acetyl cysteine on GSH content of brain of adult rats. Experientia 1983 39, 164-165.
470. Vina J ; Saez GT ; Wiggins D ; Roberts AFC ; Hems R ; Krebs HA. The effect of cysteine oxidation on isolated hepatocytes. Biochem. J. 1983 212, 39-44.
471. Vos O ; Budke L ; Fatome M ; Van Hooidonk C. Radioprotection by thiazolidines at the cellular level. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 1981 39, 291-296.

472. Wahllander A ; Soboll S ; Sies H ; Linke I ; Muller M. Hepatic mitochondrial and cytosolic glutathione content and the sub-cellular distribution of GSH-S-transferases. F.E.B.S. Lett. 1979 97, 138-140.
473. Ward WF. Radiation induced pulmonary arterial perfusion defects modification by D-penicillamine. Radiology 1981 139, 201-204.
474. Ward WF ; Molteni A ; Ts'ao C-H ; Solliday NH. Radiation injury in rat lung. IV. Modification by D-penicillamine. Radiat. Res. 1984 98, 397-406.
475. Ward WF ; Shih-Hoellwarth A ; Johnson PM. Survival of penicillamine-treated mice following whole-body irradiation. Radiat. Res. 1980 81, 131-137.
476. Ward WF ; Shih-Hoellwarth A ; Peerlman HG ; Kepka AG. Whole thorax radiation lethality in penicillamine treated mice. Radiat. Res. 1982 90, 321-329.
477. Watts ME ; Maughan RL ; Michael BD. Fast kinetics of the oxygen effect in irradiated mammalian cells. Int. J. Radiat. Biol. 1978 33, 195-199.
478. Weiss H ; Ling CC ; Epp ER ; Santomassso A ; Heslin JM. Irradiation of Serratia marcescens by single and double pulses of high-intensity electrons: Oxygen diffusion kinetics and lifetime of oxygen sensitive species. Radiat. Res. 1975 61, 355-365.
479. Welch WJ ; Garrels JI ; Thomas GP ; Lin J J-C ; Feramisco JR. Biochemical characterization of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca^{2+}-ionophore-regulated proteins. J. Biol. Chem. 1983 258, 7102-7111.
480. Wendel A ; Cikryt P. The level and half-life of glutathione in human plasma. F.E.B.S. Lett. 1980 120, 209-211.
481. Whillans DW. A rapid-mixing system for radiobiological studies using mammalian cells. Radiat. Res. 1982 90, 109-125.
482. Whillans DW. Mechanisms of oxygen radiosensitization in CHO cells. In: "Oxygen and Oxy-Radicals in Chemistry and Biology," (MAJ Rodgers ; EL Powers, eds.) 1981, pp.277-284. Academic Press, New York.
483. Whillans DW ; Hunt SW. A rapid mixing comparison of the mechanisms of radiosensitization by oxygen and misonidazole in CHO cells. Radiat. Res. 1982 90, 126-141.
484. Willson RL. Free radical repair mechanisms and the interactions of glutathione and vitamins C and E. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.1-22. Academic Press, New York.
485. Wolters H ; Konings AWT. Radiation effects on membranes. III. The effect of x-irradiation on survival of mammalian cells substituted by polyunsaturated fatty acids. Radiat. Res. 1982 92, 474-482.
486. Wright EA. Chemical protection at the cellular level. In: "Radiation Effects in Physics, Chemistry and Biology," (M Ebert ; A Howard, eds.) 1963, pp.276-289. North Holland Publ. Co., Amsterdam.
487. Wright EA. The influence of combining hypoxia and cysteamine treatments on whole body irradiation of mice. Br. J. Radiol. 1962 35, 361.
488. Yonezawa M ; Katoh N ; Takeda A. Restoration of radiation injury by ginseng panax-ginseng. 2. Some properties of the radioprotective substances. J. Radiat. Res. 1981 22, 336-343.
489. Yuhas JM. Biological factors affecting the radioprotective efficiency of S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721): $LD_{50}(7)$ doses. Radiat. Res. 1971 47, 526-529.

490. Yuhas JM. Protective drugs in cancer therapy: Optimal clinical testing and future directions. Int. J. Radiat. Oncol. Biol. Phys. 1982 8, 513-517.
491. Zajac J-M ; Bernard P. Effects of cysteamine and of irradiation of microsomal membrane bound enzymes. Enzyme 1982 28, 382-386.
492. Zalesna G ; Kopff J ; Wdzieczak J ; Wawrzyniak M ; Leyko W. Studies on the effect of superoxide dismutase on the radiation-induced changes in the DNA molecule. 3. Studies on DNA strand breaks and deoxyribose damages under aerobic and anaerobic conditions. Stud. Biophys. 1981 82, 229-234.
493. Zherebchenko PG ; Airapetyan GM ; Krasnykj IG ; Suvorov NN ; Shevchenko AN. The effect of radioprotective substances on the Neutral Red distribution and hemoglobin content in organs of mice and rats. Radiobiologiya 1964 4, 136-143.
494. Ziegler DM ; Duffel MW ; Poulsen LL. Studies on the nature and regulation of the cellular thiol: Disulphide potential. Ciba Foundation Symposium, Sulfur in Biology. 1980 72, pp.191-204.
495. Ziegler DM ; Poulsen LL ; Richerson RB. Oxidative metabolism of sulfur-containing radioprotective agents. In: "Radioprotectors and Anticarcinogens," (OF Nygaard ; MG Simic, eds.) 1983, pp.191-202. Academic Press, New York.

DISTRIBUTION LIST

Federal Emergency Management Agency
Attn: Assistant Associate Director
for Research
National Preparedness Programs
500 C Street S.W.
Washington, D.C. 20472 (3 copies)

Federal Emergency Management Agency
Attn: David W. Bensen
Office of Research
National Preparedness Programs
500 C Street S.W.
Washington, D.C. 20472 (38 copies)

Assistant Director
Energy and Natural Resources
Office of Science and Technology Policy
Executive Office Building
Washington, D.C. 20500

Office of Joint Chiefs of Staff
Pentagon
Washington, D.C. 20301

Director
Defense Nuclear Agency
Attn: Col. R. Walker/Thomas Kennedy
Washington, D.C. 20305 (2 copies)

Assistant Secretary of the Army (RD&A)
Attn: Deputy ASA for (RD&S)
Washington, D.C. 20310

Chief of Engineers
Department of Army
Attn: DAEN-RDZ-A
Washington, D.C. 20314

Chief of Naval Research
Washington, D.C. 20360

Central Intelligence Agency
Attn: CRS/DSB/IAS (Ms. Doris Lohmeyer)
Washington, D.C. 20505

Defense Intelligence Agency
Attn: WDB-4C2
Mr. Carl Wiehle
Washington, D.C. 20301

Director
Office of Military Application,
DP226.2
Department of Energy
Attn: Mr. Thaddeus Dobry
Washington, D.C. 20545

Chief, Emergency Planning Staff
Office of the Secretary of the
Treasury
Washington, D.C. 20220

National Academy of Sciences
(JH-312)
2101 Constitution Avenue, N.W.
Washington, D.C. 20418

COPRA
OJCS/SAGA
Pentagon
Washington, D.C. 20301

Headquarters USAF (SAMI)
Attn: H.A. Quinn
Pentagon 10384
Washington, D.C. 20330

Chief, National Military Command
Systems Support Center
(Code B210)
Pentagon
Washington, D.C. 20310

Industrial College of the Armed
Forces
Washington, D.C. 20319

President Naval War College
Attn: Code 1212
Newport, RI 02940

Command and Control Technical
Center
Department of Defense
Pentagon
Washington, D.C. 20301

Sandia National Laboratories
Attn: Milton R. Madsen
Division 9214
Albuquerque, NM 87185

DISTRIBUTION LIST

Oak Ridge National Laboratory
Attn: Emergency Technology Division
Librarian
P.O. Box X
Oak Ridge, TN 37830

Oak Ridge National Laboratory
Attn: Conrad Chester
P.O. Box X
Oak Ridge, TN 37830

Los Alamos Scientific Laboratory
Attn: Document Library
P.O. Box 1663
Los Alamos, NM 87544

U.S. Army Combined Arms Combat
Development Activity
Fort Leavenworth, KS 66027

Air Force Weapons Laboratory
Attn: SUL Technical Library
Kirtland Air Force Base
Albuquerque, NM 87117

Director, U.S. Army Ballistic
Research Laboratory
Attn: Document Library
Aberdeen Proving Ground, MD 21006

Director, U.S. Army Ballistic
Research Laboratory
Attn: Mr. William Taylor
Aberdeen Proving Ground, MD 21005 (2 cpy)

Director, U.S. Army Engineer
Waterways Experiment Station
Attn: Document Library
P.O. Box 631
Vicksburg, MS 39180

Director, U.S. Army Engineer
Waterways Experiment Station
Attn: W.L. Huff
P.O. Box 631
Vicksburg, MS 39180

National Bureau of Standards
Attn: Lewis V. Spencer
Center for Radiation Research
Building 245, Room C-313
Washington, D.C. 20234

Technical Library
U.S. Dept. of Energy.
Attn: Barbara Burroughs
Washington, D.C. 20545

International Association of
Chiefs of Police
Attn: Bjorn Pedersen
11 Firstfield Road
Gaithersburg, MD 20760

National Defense Transportation
Association
Attn: Gerald W. Collins
1612 K Street, N.W. - Suite 706
Washington, D.C. 20006

The Council of State Governments
Disaster Assistance Project
1225 Connecticut Ave., N.W.
Suite 300
Washington, D.C. 20036

Director, Council of State
Governments
Attn: Leo A. Hoegh
Timpa Road
Chipita Park, CO 80811

Defense Technical Information
Center (DTIC)
Cameron Station
Alexandria, VA 22314 (12 copies)

DISTRIBUTION LIST

U.S. Army Training & Doctrine Command
Fort Monroe
Hampton, VA 23651

Director
USAMC Intern Training Center
Red River Army Depot
Attn: AMXMC-ITC-L
Texarkana, TX 75501

Institute for Defense Analyses
Program Analysis Division
Attn: Leo A. Schmidt
1801 N. Beauregard Street
Alexandria, VA 22311

Analytical Assessments Corporation
Attn: Howard Berger
P.O. Box 9758
Marina del Rey, CA 90291

Analytical Sciences Corporation
Attn: Gen. L. Bray
1601 North Kent Street
Suite 1201
Arlington, VA 22209

Boeing Company
MASD Library
Attn: R.E. Shipp 23-99
P.O. Box 3955
Seattle, WA 98124

Calspan Corporation
Attn: Donald Drzewiecki
P.O. Box 400
Buffalo, NY 14225

Center for Planning and Research, Inc.
Attn: Walmer E. Strope
5600 Columbia Pike, Suite 101
Bailey's Crossroads, VA 22041

Checchi and Company
Attn: Harvey Lerner
815 Connecticut Ave., N.W.
Washington, D.C. 20006

The Dikewood Corporation
1613 University Boulevard, N.E.
Albuquerque, NM 87101

General Electric Company
Space and RESD Division (Library)
Philadelphia, PA 19104

General Research Corporation
Attn: Library/db
7655 Old Springhouse Road
McLean, VA 22101

Hudson Institute
Quaker Ridge Road
Croton-on-Hudson, NY 10520

John Hopkins Applied Physics
Laboratory
Attn: Robert Fristrom
Johns Hopkins Road
Laurel, MD 20707

Kaman Sciences Corporation
Attn: Donald Sachs
1911 Jefferson Davis Highway
Arlington, VA 22202

Director
Lovelace Foundation
5200 Gibson Boulevard, S.E.
Albuquerque, NM 87108

Mission Research Corporation
Attn: Harvey Ryland
P.O. Drawer 719
Santa Barbara, CA 93102

Ohio State University
Disaster Research Center
127-129 West 10th Avenue
Columbus, OH 43201

Physics International Company
Attn: Fred Sauer
2700 Merced Street
San Leandro, CA 94577

Pugh-Roberts Associates, Inc.
Attn: David W. Peterson
Five Lee Street
Cambridge, MA 02139

DISTRIBUTION LIST

The RAND Corporation
Attn: Document Library
1700 Main Street
Santa Monica, CA 90406

R&D Associates
Attn: Dennis Holliday
P.O. Box 9695
Marina del Ray, CA 90291

Research Triangle Institute
Attn: Donald Johnson
P.O. Box 12194
Research Triangle Park, NC 27709

Ryland Research, Inc.
Attn: Harvey G. Ryland
5266 Hollister Avenue, Suite 324
Santa Barbara, CA 93111

Science Applications, Inc.
Attn: Marvin Drake
1200 Prospect Street
La Jolla, CA 92037

S-Cubed
Attn: Russell Duff
Charles Needham
11800 Sunrise Valley Dr.
Reston, VA 22091

SRI International
Attn: Richard Foster
1611 Kent Street
Arlington, VA 22209

Systan, Inc.
Attn: John Billheimer
P.O. Box U
Los Altos, CA 94022

The Center for Strategic and
International Studies
1800 K Street, NW
Washington, DC 20006

System Development Corporation
Attn: Murray Rosenthal
2500 Colorado Avenue
Santa Monica, CA 90406

Systems Planning Corporation
Attn: Leonard Sullivan, Jr.
1500 Wilson Boulevard
Suite 1500
Arlington, VA 22209

Technology & Management
Consultants
300 Washington Street
Suite 613
Marina del Ray, CA 90291

Director, Institute for Disaster
Research
College of Engineering
Attn: Joseph E. Minor
Texas Tech University
P.O. Box 4089
Lubbock, TX 79409

Department of the Aerospace and
Engineering Sciences
Attn: Forman Williams
University of California
San Diego
La Jolla, CA 03027

Underwriters' Laboratories, Inc.
Attn: William F. Christian
333 Pfingsten Road
Northbrook, IL 60062

DISTRIBUTION LIST

Dr. Vilhelm Sjolin
Director of BRANDFORSK
The Swedish Fire Research Board
S-115 87 Stockholm
SWEDEN

Secretaire d'Administration
Ministere de l'Interieur
Direction Generale de la
Protection Civile
rue de Louvain, 1
1000 Brussels
BELGIUM

Canadian Defence Research Staff
Attn: Dr. K.N. Ackles
2450 Massachusetts Avenue, N.W.
Washington, D.C. 20008

Director
Civilforsvarsstyrelsen
Stockholmsgade 27
2100 Copenhagen 0
DENMARK

Direction 'de la Securite Civile
Ministere de l'Interieur
18 Rue Ernest Cognac
92 Levallois (Paris)
FRANCE

Bundesministerium des Innern
Graurheindorfer Strasse 198
5300 Bonn 1
WEST GERMANY

Ministry of Social Services
11 Spartis Street
Athens
GREECE

Almannavarnir Rikisins
Reykjavik
ICELAND

Stato Maggiore Difesa Civile
Centro Studi Difesa Civile
Rome
ITALY

Civil Emergency Planning
Directorate
North Atlantic Treaty Organization
1110 NATO
BELGIUM

Jefe, Seccion de Estudios y
Planification
c/Evaristo San Miguel, 8
Madrid-8 SPAIN

Ministero dell Interno
Direzione Generale della
Protezione Civile
00100 Rome
ITALY

Directeur de la
Protection Civile
Ministere de l'Interieur
36 Rue J.B. Esch
GRANDE-DUCHE DE LUXEMBOURG

Directeur Organisatie
Bescherming Bevoling
Ministry of Interior
Schedeldoekshaven 200
Postbus 20011
2500 The Hague
NETHERLANDS

The Head of Sivilforsvaret
Sandakerveien 12
Postboks 12
Oslo dep
Oslo 1
NORWAY

Servico National de
Proteccao Civil
Rua Bela Vista a Lapa, 57
1200 Lisbon
PORTUGAL

Civil Defense Administration
Ministry of Interior
Ankara
TURKEY

Home Office
Scientific Advisory Branch
Horseferry House
Dean Ryle Street
London SW1P 2AW
ENGLAND

Dr. Ing. P.G. Seeger
Forschungsstelle fur
Brandschutztechnik
University of Karlsruhe (TH)
75 Karlsruhe 21
Postfach 6380
WEST GERMANY